STRATEGIES FOR SMALL-SCALE PRODUCTION OF DRIED APPLES AND HUMMUS PRODUCTS WITH NATURAL INGREDIENTS

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ABSTRACT

Hummus, a Middle-Eastern legume-based spread, is increasingly popular in the United States, but science-based guidelines to ensure product safety and quality are lacking. Preservatives are used to extend hummus' refrigerated shelf life, but consumers prefer natural alternatives. We evaluated the effect of natural antimicrobials and acid systems 1) on the shelf life of refrigerated hummus and 2) in combination with thermal processing as a hurdle technology to develop a shelf-stable product. Base hummus was prepared by blending chickpeas, olive oil, salt, and water. Hummus was acidified to pH 4.6 with citric acid, citric and acetic acids, glucono-delta-lactone (GDL), or GDL and acetic acid. Five preservative treatments were tested: 20 ppm natamycin, 500 ppm and 1000 ppm natural plant bitters, 1000 ppm potassium sorbate, and no antimicrobial (control). Samples were inoculated with *Penicillium*, stored at 15°C for 5 weeks (accelerated study), and analyzed weekly for microbial counts. Following the accelerated study, the citric-acetic acid system was validated for storage at 5°C with 20 ppm natamycin, 1000 ppm potassium sorbate, 1000 ppm sodium benzoate, and no antimicrobial at pH 4.6 and 4.2. Samples were inoculated and analyzed as previously described.

Over the accelerated study, total plate counts and yeast and mold counts were lowest in citric-acetic acid hummus with natamycin compared to all other samples ($p<0.05$), with >5 weeks shelf-life. Hummus with GDL or GDL-acetic acid had no significantly different microbial counts ($p>0.05$) among preservative treatments. During 5°C storage, yeast and mold counts were below the limit of detection (<1 CFU/g hummus) by Week 12 for citric-acetic hummus with natamycin, demonstrating fungicidal activity. Citric-acetic acid hummus with natamycin achieved 22 weeks of refrigerated shelf life at both pH, which was significantly longer ($p<0.01$) than the other preservative treatments.

Hot-packing trials with citric-acetic hummus at pH 4.2 were completed to produce a shelf-stable product. Fill temperatures of 76.7°C, 82.2°C, and 87.8°C were tested with 20 ppm natamycin, 1000 ppm potassium sorbate plus 1000 ppm sodium benzoate, and no antimicrobial. Hummus was inoculated with *Penicillium* and *Aspergillus niger* prior to heat treatment. Samples were analyzed post-thermal treatment for initial microbial enumeration. Incubation trials at 35°C were conducted to determine if products were shelf-stable. A shelf-stable citric-acetic hummus at pH 4.2 could be produced if hot-filling at ≥87.8°C without preservatives. Hot-filling at ≥82.2°C with natamycin achieved shelf stability, while hot-filling at ≥76.7°C was adequate with potassium sorbate plus sodium benzoate. These treatments had no mold growth and no significant increase in bacterial counts after the 10-day incubation period.

The acceptability of the low-browning apple variety Autumn Crisp in drying applications was investigated to achieve a clean label, minimally processed dried product. Autumn Crisp apples were peeled, cut into wedges, and dried by convective heating without pre-treatments or the use of sulfites. The resultant product was not significantly different in brown color or instrumental texture analysis from a commercial product made with sulfites ($p>0.05$). A consumer acceptability test was conducted to compare acceptance of the Autumn Crisp product to two commercial products, one with and one without sulfites. Dried apple products made from the Autumn Crisp variety were as acceptable as commercial sulfited products in terms of flavor, texture, color, and overall liking. There was no significant difference in consumers' willingness-to-pay between Autumn Crisp dried apple products and commercial products with sulfites ($p>0.05$), indicating strong market potential for this apple variety in the dried fruit category.

The information from these studies will be essential in helping small-scale producers develop scheduled processes for safe and high-quality products.

BIOGRAPHICAL SKETCH

Claudia grew up in Belle Mead, NJ in a household with Peruvian and Ecuadorian roots. Her interest in food science stemmed from a love of international cuisines and an awareness of food additives with her mother's sensitivities to sulfites. She attended Cornell University in Ithaca, NY to explore these questions and graduated with a B.S. in Food Science, Summa Cum Laude with Distinction in Research, in 2012. During her undergraduate career, Claudia was part of several product development teams, interned for Unilever in the U.S. and the U.K., and studied abroad at the Universitat Autònoma de Barcelona. She was recognized in 2012 with the SUNY Chancellor's Award for Student Excellence and as a Merrill Presidential Scholar.

Eager to continue her education, Claudia continued straight through to graduate school at Cornell, completing her research at the New York State Agricultural Experiment Station in Geneva, NY. As a master's student, she was a teaching assistant for FDSC 1102 and FDSC 1500, an extension assistant at the Food Venture Center, and co-chair of social activities with the Student Association of the Geneva Experiment Station (SAGES). Claudia interned with the research and development unit at General Mills during her summer, and upon graduation, she will join the company full-time at their headquarters in Minneapolis, MN.

To Hope Bertelsen

True supertaster, fellow hummus lover, and extraordinary friend

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CHAPTER 1
INTRODUCTION TO HUMMUS PRODUCTION

1.1 Background

Hummus is a traditional Middle-Eastern dip made from cooked chickpeas mashed with tahini (sesame paste) and typically flavored with lemon juice, salt, and garlic (Al-Holy et al. 2006). With the growing popularity of ethnic cuisines in the United States, hummus consumption has increased rapidly over the past few years, totaling $530 million in sales in 2012 and having increased 25% from 2010 (Kesmodel & Fletcher 2013). Consumers associate hummus with the health benefits of a Mediterranean diet (Browne 2011), being higher in protein and lower in fat than many other spreads or dips. The Refrigerated Spreads category as a whole has reached $878 million, showing a 13% three-year annual compound growth rate (Sargento Foods 2013) and providing opportunity for many new products to succeed in the market. With the expansion of the market has come large-scale commercialization of hummus products, including brands like Sabra®, Tribe®, Athenos®, and private-label names. However, like other ethnic foods, consumers may prefer a less industrialized hummus, providing an advantage to smaller food producers that offer an authentic and traditional product.

There is currently no standard of identity for hummus in the United States, leading to many variations of formulations with different spices and flavors. Reported pH values for commercial products range from 4.50-6.30, depending on the added ingredients, and water activity is generally high (0.975-0.992). Hummus presents food safety risks, as the raw ingredients can introduce different microorganisms and it is not traditionally heat-treated (Yamani & Al-Dababseh 1994). At the Cornell University Food Venture Center, hummus products are only approved by the process authority if pH ≤ 4.6 for refrigerated products, or pH ≤ 4.2 for shelf-stable products. These pH limits are set for food safety, as pH ≤ 4.6 protects against

Clostridium botulinum growth and other pathogenic microorganisms. In the United Arab Emirates (UAE), regulations state that the shelf life of ready-to-eat (RTE) salads, including hummus, should not exceed one day to ensure that bacterial counts are less than 10^5 CFU/g (Almualla et al. 2010). Quality issues with hummus are often mold-related, as a product with mold growing on the surface will prompt a negative consumer reaction even if the bacterial counts are below 10^5 CFU/g. Gaining a better understanding of the microbial loads and potential sources of contamination with hummus is the first step to improving the safety and quality of this product.

1.2 Food Safety and Quality of Hummus

There has been limited scientific research completed on hummus, but of those studies published, multiple have indicated that hummus poses a food safety risk. Yamani and Al-Dababseh (1994) analyzed 60 hummus samples from fifteen different Jordanian restaurants for pathogenic and indicator microorganisms. Samples were compared to a reference lab-prepared hummus sample. Average counts of aerobic plate counts (APC), lactic acid bacteria (LAB), and coliforms were 1.9×10^8, 1.6×10^8, and 2.9×10^5 CFU/g, respectively, during the summer. These counts were significantly lower ($p < 0.05$) in the winter: 2.7×10^7, 1.6×10^7, and 2.2×10^3 CFU/g for APC, LAB, and coliforms, respectively. Yeast counts were at 10^4 CFU/g in both seasons. The high levels of microorganisms indicate that hummus is a viable environment for bacteria and fungi. It is likely that much of the contamination came during mishandling in preparation, as the lab reference sample had much lower counts ($<10^3$ CFU/g for APC & LAB, $<10^2$ for yeast & coliforms) and was prepared in known hygienic conditions. Although no pathogens were isolated from these samples, the authors stated that it does not preclude hummus from being a vehicle for such organisms. The results of the study indicate the importance of

current good manufacturing practices (cGMPs) during product preparation, the impact of storage temperature conditions, and the need for more pathogen viability testing in hummus.

A more recent study by Almualla et al. (2010) investigated the microbial load of RTE hummus from three supermarket delis in the UAE. Aerobic plate counts were 10^4 CFU/g after only one day of storage at 5°C and surpassed 10^5 CFU/g after two days. No *S. aureus*, *L. monocytogenes*, or *Salmonella* were detected in the samples. Although *E. coli* was isolated from the hummus samples, the counts were low (0.5 log CFU/g) and it was not confirmed that the isolated strain was pathogenic. The authors concluded that the high aerobic counts were of concern and corroborated the UAE law that RTE products, including hummus, have a recommended shelf life of one day. Such high counts in refrigerated hummus products are concerning, as refrigerated storage is often a safety measure employed in food distribution.

Researchers in Saudi Arabia noted high microbial loads in hummus as well, with samples from local restaurants having total aerobic bacteria counts of 2.5×10^5 CFU/g and total coliform counts of 8×10^4 CFU/g (Khiyami et al. 2011). In comparison to the restaurant samples, homemade hummus collected from selected households had slightly lower total aerobic and total coliform counts, 7.9×10^4 CFU/g and 3.2×10^3 CFU/g, respectively. One restaurant hummus sample was also found to contain *Shigella* and *Salmonella* species. These results further confirm the food safety risk associated with a minimally processed food like hummus and the need to educate food handlers, both at home and in commercial settings.

Epidemiological reports have also implicated hummus to be a potential food safety risk. A case-control study by Varma et al. (2007) of sporadic listeriosis cases found that *L. monocytogenes* infection was associated with eating hummus prepared from a commercial establishment (odds ratio 5.7, 95% confidence interval 1.7-19.1), using data from FoodNet

during 2000 to 2003. Eating hummus in general was also associated with an increased likelihood of *L. monocytogenes* serotype 4b infection (OR 3.19, 95% CI 0.98-10.33). In addition, a multistate outbreak of *Salmonella* serotype Bovismorbificans in 2011 was ultimately linked to hummus and tahini (Mortality and Morbidity Weekly Report 2012). From open-ended interviews with the 23 case patients, it was determined that three Mediterranean restaurants in the Washington D.C. area were connected to the outbreak. Testing of the hummus and hummus ingredients (including tahini) were found to be positive for *S*. Bovismorbificans, with its DNA identification indistinguishable from the outbreak strain. Traceback by the FDA later suggested that the tahini used in the hummus was the plausible source for *Salmonella* infections, as it came from a Lebanese manufacturer associated with recent *Salmonella* outbreaks in Canada.

The source of the microbiological contamination may be from food handlers and the processing environment, but it is also possible that the contamination is from an ingredient. Tahini is recognized as a high-risk food, with one study finding 32.5% of 120 tahini halva samples to not meet the Turkish Food Codex for safety (Kahraman et al. 2010). Three outbreaks of *Salmonella* Montevideo in Australia and New Zealand from 2002-2003 were also connected to tahini, leading to an international investigation of the safety of sesame-based products (Unicomb et al. 2005). Recently in the U.S., hummus was recalled due to potential *Listeria monocytogenes* contamination from the fresh green chiles used for flavor (FDA 2014), indicating additional risk from other contaminated raw ingredients.

As these research reports and product recalls indicate, hummus presents both food safety and food quality issues. The high microbial load in hummus confirmed by Almualla et al. (2010) and Khiyami et al. (2011) is of concern for manufacturers who wish to sell and distribute hummus, as it severely impacts the quality and shelf life. Both the case-control study and the

multiple outbreaks highlight the need to provide science-based guidelines on formulation and processing to hummus manufacturers so that safe products can be supplied. Although these studies demonstrated the majority of food safety concerns to be in retail establishments, small-scale producers will likely encounter similar problems, as their operations are similar in size.

1.3 Formulation Hurdles for Hummus Manufacturing

To improve microbial safety and extend the shelf life of hummus, there are changes that can be made to the formulation of the food product. With formulation, modifying water activity, addition of antimicrobials, and different acid systems for lowering pH all offer potential advantages. Several studies have been conducted in each area with various degrees of success, which are reviewed below.

1.3.1 Water Activity Modification

With the water activity of hummus being so high (generally over 0.98), it serves as an excellent growth medium for microorganisms. A potential method to control microbial growth is lowering the water activity. One study by Alali et al. (2012) investigated the survival and growth characteristics of *Salmonella* and *L. monocytogenes* in hummus as affected by sodium content, which effectively changes the water activity. Hummus was inoculated with a 5-serotype strain of *Salmonella* and another set of hummus samples were inoculated with a 5-strain broth of *L. monocytogenes*. An addition of 265 to 728 mg NaCl/100 g (41.1% - 113% of control NaCl) was used in the samples, resulting in Aw values from 0.975 to 0.992. While neither *Salmonella* nor *L. monocytogenes* grew in the hummus samples, the pathogens did survive at all temperatures and sodium concentrations. Mean *Salmonella* populations were significantly ($p < 0.05$) lower in hummus containing the lowest sodium concentration (i.e. no sodium added), regardless of storage temperature, compared with hummus containing added sodium. However, the numerical

differences were quite small (0.2-0.3 log CFU/g) and thus may not be biologically significant. Upon comparing the hummus results with other foods sampled, it was determined that the acidic pH of the hummus (pH 4.5-4.59) was a major factor in preventing growth of both pathogens. The authors concluded that additions of the sodium concentrations used in the study have little or no effect on the behavior of *Salmonella* and *L. monocytogenes* when hummus is stored at 4 or 10°C for up to 27 days. This study indicates that small changes in water activity are not sufficient to affect the microbial load of hummus, and to achieve larger differences in water activity would require much more drastic additions of salt and result in extreme changes in organoleptic quality. Therefore, water activity is most likely not a viable parameter to manipulate for improved safety or quality of hummus.

1.3.2 Addition of Acid to Lower pH

The antimicrobial effects of acidification are dependent on the type of acid used and the pH achieved. Altering the pH of the food matrix forces the microorganisms to change their metabolic activities, as microorganisms maintain a higher internal pH than their surroundings (Gould 1996). Thus, increasing the acid to lower the pH can restrict or inhibit microbial growth with the disruption of homeostasis. Lowering the pH below 4.6 can successfully inhibit *Clostridium botulinum* and other bacterial growth, as been previously discussed, but molds are typically more tolerate to lower pH values than bacteria. Although certain acids are stronger than others, i.e. less is needed to decrease the pH, only some acids, particularly weak acids, have a specific antimicrobial effect.

By the weak-acid theory, weak acids with low pK_a values (such as acetic acid and lactic acid) remain in undissociated forms even at low pH. It is easier for acids in their undissociated forms to cross the cell membrane and then dissociate, thereby lowering the internal pH of the

microbe and disrupting homeostasis (Brul and Coote 1999). Citric acid is the predominant acid in lemon juice, which is commonly used as an ingredient in hummus and helps lower the product's pH. However, citric acid does not follow traditional weak acid theory and is instead a chelator that inhibits microbial growth by binding divalent metal ions so they may no longer be used for metabolism (Brul and Coote 1999). In comparison to acetic and lactic acids, citric acid was not shown to have as strong of an antimicrobial effect against *Shigella* species (In et al. 2013), while both acetic and lactic acids have also demonstrated strong bactericidal effects against *Listeria* and *E. coli* O157:H7 (Kreske et al. 2008). This has led to multiple scientific guidelines to use acetic or lactic acids in food formulation or processing to ensure safety against pathogens (Sullivan et al. 2013, Khurana et al. 2006).

Certain organic acids have also shown strong antifungal effects. Lind et al. (2005) investigated the effect of propionic, acetic, and lactic acids on the growth of mold and yeast species. Both propionic and acetic acid inhibited all fungal growth at pH 3 at concentrations between 4 and 30 mM, while lactic acid required concentrations from 160 mM to over 500 mM. At pH 5, minimum inhibitory concentration (MIC) levels of propionic, acetic, and lactic acid were $\leq$ 60 mM, $\leq$ 120 mM and > 500 mM, respectively. These results indicate that the metabolic byproducts propionic and acetic acids hold large potential as "natural" preservatives against yeasts and molds and are more effective at lower pH.

The strong antimicrobial effect of acetic acid was also supported in a study by Stratford et al. (2009), which found the MIC of acetic acid against several spoilage molds to be 40-110 mM at pH 4 in *Aspergillus* Complete Medium (ACM). In contrast, the MIC of sorbic acid ranged between 1.5-3.5 mM. Thus, on a molar basis, molds were 30 times more resistant to acetic acid than sorbic acid. Yet no correlation between resistance to sorbic acid and resistance

to acetic acid was found among the tested species. As an example, *Aspergillus pheonicis* was highly resistant to sorbic acid but was the most sensitive of all tested species to acetic acid, indicating that the use of acetic acid for antimicrobial effects may work better on a case-by-case basis.

However, the increased sour taste that results from lowering the pH with acids is not always desirable. Glucono-delta-lactone (GDL) is a slow-release acidulant with a mild taste, making it an appealing alternative to organic acids with sour notes. When in aqueous solution, GDL slowly hydrolyzes to gluconic acid, thereby gradually lowering the pH and resulting in a slightly acidic taste (Pszczola 2007). GDL has also demonstrated antimicrobial effects against *Listeria monocytogenes* in both frankfurters and bologna (Samelis et al. 2002, Barmpalia et al. 2005). Given the benefits of acidification and antimicrobial effects without drastically changing the taste, GDL could be a desirable acidulant for hummus production.

The advantages of acidification also extend into thermal processing. Time and temperature of heat treatment to achieve shelf-stability of food products is pH dependent, with much lower processing temperatures required for pH $\leq$ 4.2 compared to higher pH values because the target microorganisms are less viable in the acidic environment (Gould 1996). As such, achieving lower pH values in food formulations will enable use of milder heat treatment, an option that is more fully discussed in Section 1.4. Most importantly, this highlights the potential to employ pH as a hurdle technology to achieve extended shelf life while maintaining organoleptic qualities.

1.3.3 Addition of Antimicrobials

The use of antimicrobials to control the growth of bacteria, yeasts, and molds is commonly applied in commercial hummus operations. Researchers, with varying degrees of

success, have investigated applications of both chemical preservatives and "natural" preservative alternatives in hummus.

1.3.3.1 Chemical preservatives

Given the antifugal effect of sorbic acid previously discussed from Stratford et al. (2009), potassium sorbate has become a popular preservative to use in hummus to control mold growth. Potassium sorbate is the potassium salt of sorbic acid and is appreciably soluble in water. It is commonly used in hummus to increase shelf life, with 0.1% (1000 ppm) being its maximum permitted level of use in food (FDA 2013). Sodium benzoate is another chemical preservative commonly used in hummus for controlling both mold and bacterial growth, being the sodium salt of benzoic acid. Its maximum permitted level of use in food is also 0.1% (FDA 2013).

A study conducted by Yamani and Mehyar (2011) investigated the effectiveness of potassium sorbate, sodium benzoate, and sodium metabisulfite to extend shelf life of hummus prepared at pH 4.5. Samples deemed unacceptable by the sensory panelist (for off-flavors, off-odors, etc.) were categorized as spoiled. Potassium sorbate (PS) was found to have a greater inhibitory effect on yeasts and molds than sodium benzoate (SB), extending refrigerated shelf life to 35 and 49 days when used at 0.05% and 0.1%, respectively. The same shelf life could be achieved with SB, but only at higher concentrations of 0.1% and 0.15%. Sodium metabisulfite (SM) extended refrigerated shelf life to 63 and 77 days at 0.05% and 0.1%, respectively. However, the yeast and mold counts were higher with SM than the other two preservatives while *Enterobacteriaceae* counts were lower with SM. This may indicate that the microorganisms responsible for sensorial quality failure in hummus are *Enterobacteriaceae*. A combination of PS and SM produced a synergistic effect, with no spoilage noted after 90 days of storage. The

higher pK_a values for these two acids (4.75 for PS and 7.15 for SM) may attribute to their greater success in an acidified hummus compared to SB (pK_a value of 4.20).

Despite the effectiveness of chemical preservatives, there is growing consumer sentiment against chemical additions to food products. This trend presents some challenges for food processors, as consumers are increasingly demanding fresh products with an all-natural "clean label" but still with a convenient, long-lasting shelf life (Zink 1997). To meet the consumers' expectations, new formulations including novel antimicrobials are being trialed across multiple food categories, including hummus.

1.3.3.2 Microbial-Sourced Antimicrobials

One such natural antimicrobial is nisin, a lantibiotic bacteriocin produced by *Lactococcus lactis* that is effective against gram-positive vegetative bacteria and spores of *Clostridium* and *Bacillus* species (Davidson et al. 2013). It is currently approved for use in the U.S. for cheese products at a level of 250 ppm (21 CFR 184.1538). Its application in hummus was trialed by researchers investigating the inhibition of *L. monocytogenes* with varying concentrations of nisin and citric acid (Al-Holy et al. 2006). Treated hummus was inoculated with a *L. innocua* cocktail at a concentration of 10^6 CFU/g and stored at 4°C for 15 days. Neither nisin nor citric acid alone were effective in reducing the *L. innocua* growth rate for an extended period of time, as the growth rate increased again after two days once cells had recovered from initial treatment. A combination of 1000 IU/g nisin and 0.3% citric acid was the most effective in reducing *L. innocua* (approximately 2-log reduction) for 9 days, but counts then increased again by Day 12. The moderate success of this treatment may have been due to the lower pH obtained (pH 5.32 compared to pH 6.28 of control). The synergistic effect between citric acid and nisin that was observed in brain-heart infusion broth during preliminary tests was not observed in the hummus,

emphasizing the need to conduct product-specific microbial tests. The authors concluded that the nisin-citric acid combination of 1000 IU/g and 0.3% could be used to control *Listeria* growth but should not be substituted for general sanitation practices and cGMPs. However, the increase of *Listeria* growth after an initial decrease indicates that some bacteria are nisin-resistant and that nisin is not broadly applicable. Moreover, given the limited lasting effect of nisin and citric acid (only 9 days), this treatment does not hold much promise for extending the shelf life of hummus.

Singh et al. (2001) also investigated the inhibition of *L. monocytogenes* with nisin, this time pairing it with aqueous garlic extract (AGE). AGE had been previously demonstrated to have some antimicrobial effect from its active compound allicin, a di-allyl thiosulfinate (Unal et al. 2001). Minimum inhibitory concentrations (MIC) of nisin + AGE were first determined in TPB at varying pH values, and MIC values were found to increase with increasing pH. Hummus was then inoculated with 10^6 CFU/g *L. monocytogenes* and treated with sub-MIC nisin+AGE levels. While the preservatives inhibited Listeria growth in all cases, the best inhibition was achieved with 10 mg nisin + 10 g AGE per kg hummus (approximately 0.85 log reduction). The authors concluded that AGE improved the efficacy of nisin in inhibiting growth of *L. monocytogenes*, but the degree of success is dependent on pH. However, in food preservation, a reduction of <1 log in bacterial growth may not be significant for practical use nor shelf life extension. From these two studies of nisin applications in hummus, this antimicrobial does not seem to have substantial success from a food safety or quality standpoint.

Another natural antimicrobial not yet trialed in hummus is natamycin (also known as pimaricin), a polyene macrolide antimycotic produced by *Streptomyces natalensis* during fermentation (structure exhibited in **Figure 1.1**). It is most effective at inhibiting fungal growth, in which it interacts with ergosterol in the plasma membrane and blocks vacuole fusion (te

Welscher et al. 2010). It is currently approved for use in cheese at levels of 20 ppm in the U.S. (21 CFR 172.155), and it is available commercially under multiple trade names, including Natamax® (Dupont Chemicals, New Century KS) and Natadex® (Handary, Brussels, Belgium).

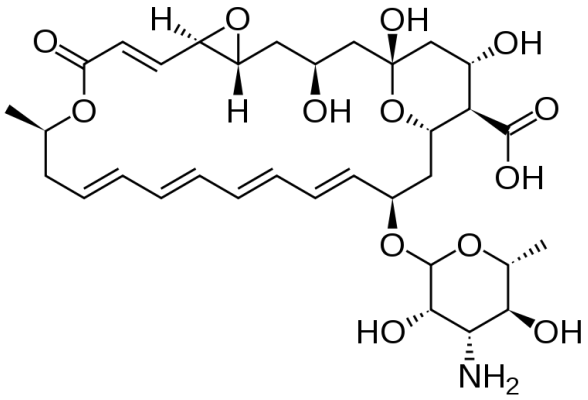

Figure 1.1 Structure of natamycin

As it is not active against bacteria, natamycin has been studied for use in fermented foods where specific antimicrobial activity is desired. Given its low solubility in water (40 μg/mL), natamycin has been most commonly used as a surface treatment. A study of antimicrobial films found that films containing natamycin (2 mg/10 g film) was inhibitory against *Aspergillus niger* growth on the surface of kashar cheese for 30 days (Ture et al. 2011). However, the same level of treatment was not inhibitory against surface growth of *Penicillium roqueforti*. Another study investigated the application of natamycin during black olive fermentation (Hondrodimou et al. 2011). Inclusion of 0.01% (w/v) natamycin in the olive brine inhibited growth of yeasts and growth of fungal mycelia on the brine surface for 60 days. The authors concluded that natamycin is an effective preservative at low concentrations in black olive fermentation to inhibit mold and yeast growth, though further research must be completed to optimize the conditions.

From these studies discussed, there is evidence that natamycin may offer desired protection against mold spoilage in acidified hummus, where the pH is already low enough to inhibit growth of pathogenic bacteria. Although natamycin has low water solubility, it could

easily be incorporated with other dry ingredients to be best dispersed through the product. Its low level of inclusion would also not change the taste or flavor of the hummus. Additionally, natamycin is heat-stable, making it a viable preservative in thermal processing applications. Although it is not currently approved for use in hummus, the GRAS status of natamycin could be extended to include applications in legume-based spreads. No published research has investigated the efficacy of natamycin against spoilage in hummus, which would help justify the GRAS status extension.

1.3.3.3 Plant-Based Antimicrobials

Certain plant extracts also exhibit antibacterial and antifungal activity, and these compounds can be considered natural preservative ingredients as well. Many of the antimicrobial agents are phenolic compounds believed to be secondary metabolites produced for protection against insects and phytopathogens (Davidson et al. 2013). Examples of compounds with studied antimicrobial effects include carvacrol from oregano and allicin from garlic, which are active against both bacteria and fungi. One commercial product is plant bitters extract, which has shown to inhibit mold growth in jams of 55° Brix at levels of 250 ppm (Churey 2012, personal communication) and may have broader applications. Use of plant ingredients is advantageous as they are recognizable to consumers as being natural, thereby allowing a "clean label." However, addition of such ingredients may also impact flavor or aroma, which must be considered during product formulation. These products can also be very expensive, and the quantity required to inhibit microbial spoilage is not always specified.

1.4 Thermal Processing Options for Hummus Manufacture

Thermal processing alternatives can be applied to enhance safety and quality of hummus. In the U.S. market, many of the large commercial brands are able to achieve refrigerated shelf

life of approximately 60 days, with only potassium sorbate as a preservative and citric acid as an acidulant. This shelf life is achieved because the hummus is also thermally pasteurized using high-temperature short-time (HTST) regimes. Such a process will eliminate the pathogenic bacteria and many of the spoilage microorganisms, thereby extending the shelf life. However, thermal treatment may change flavor and texture, and it does not meet the consumers' perception of an "all-natural" and "fresh" product. One new development is the application of high pressure processing (HPP) with hummus for extension of refrigerated shelf life. HPP is a non-thermal application that employs high pressure (>100 MPa) to inactivate microorganisms and enzymes in the food product (Rastogi et al. 2007). This processing option is very attractive to manufacturers of "all-natural" products that wish to avoid any preservatives on the label and still maintain a fresh taste while having a shelf life greater than eight weeks (Reynolds 2014). However, the equipment required for HPP is costly and may not be feasible for small-scale producers. For small manufacturers looking to produce a refrigerated hummus product, thermal processing or HPP may not be the ideal solution.

For shelf-stable hummus products, thermal treatment is required but also must be considered with other factors. The popularity of hummus with U.S. consumers indicates that there is market opportunity for hummus products in the shelf-stable category (Perkowski 2014). Producing a shelf-stable product would require some form of processing, usually involving heat, to eliminate microorganisms that could grow and spoil the product. There are several processing options available that may be applied manufacture a shelf-stable hummus product.

1.4.1 Processing Options for Shelf-Stable Hummus

In the Middle East, it is not uncommon to find shelf-stable canned hummus products, which greatly increases product shelf life through thermal processing that eliminates pathogenic

and spoilage microorganisms. A study by Amr and Yaseen (1994) determined that recommended process requirements for small- and medium-size cans filled with hummus to be 32.9 and 57.2 min, respectively, at 121°C, with a filling temperature of 85°C, using F_0 = 2.78 min. These temperature values are used as reference for regulatory agencies and for processors retorting low-acid hummus (pH > 4.6). While retorted processes do improve the safety of hummus, it does not satisfy consumer expectations for a "fresh" or "minimally-processed" product. Specific organoleptic qualities would also be of concern after processing at 121°C for over a half-hour, should the researchers' recommendations be applied during manufacturing.

Aseptic processing heats food at very high temperatures (93°C to 140°C depending on pH) for a few seconds (2 to 15 s) and then directly fills the food into sterile packages, resulting in shelf-stable products with relatively long (>2 years) shelf life. This processing regime has recently been applied to hummus with the Wild Garden Hummus To Go product, a 3.1-oz. serving of hummus processed and packaged into an aseptic tetrahedral TetraPak that requires no refrigeration until after opening (Jed 2012). Because the high heat treatment is for such a short period of time, organoleptic changes are minimal. This product is currently produced in Jordan and imported into the U.S., and it is available on airline meals and in supermarkets. Unfortunately, aseptic processing equipment is a very expensive investment, and most companies would likely have to sub-contract the manufacturing to a co-packer. Expensive energy costs are also of concern with aseptic processing and retort methods of preservation, especially for small-scale producers. If lower temperatures could be used in combination with other hurdles to achieve shelf-stability, thermal processing hummus would be more accessible to smaller producers and potentially more appealing to consumers.

Another thermal treatment option for hummus products with pH < 4.6 is known as "hot fill/hold", a process commonly used for acidified foods. This method involves heating the product, filling the hot product into the final containers, sealing the containers, and holding at a sufficiently high temperature to ensure that the temperature throughout the product is at or above the minimum temperature prescribed once the closure is sealed. The container is often inverted for a defined period of time (typically three to five minutes) to heat the top of the closure and is then cooled. The heat from the product must be enough to also heat the container so that the package is sterilized as well (GMA-SEF 2007a). Typically, the amount of headspace in the container is also specified, and the entire process must be reviewed and approved by the process authority. However, the hot-fill hold method is only applicable to foods that can be pumped directly into the finished containers. Hummus is a viscous product but is pumped successfully with the proper pumping equipment, such as piston fillers. Hummus also decreases in viscosity with increased temperatures, which could aid with pumping the heated hummus product. Furthermore, the hot-fill hold method is feasible for small batch sizes and is therefore appealing to small-scale producers who do not have the economic support to invest in larger processing equipment like retorts or specialized pasteurizers.

1.4.2 Thermal Processing Equipment – Scraped Surface Heat Exchangers

With hummus being a very viscous product with high water content, heating large amounts of hummus in an open kettle results in scorching and water loss from evaporation. A better alternative to the open kettle for heating could be a closed steam kettle with scrapped surface agitation or a continuous scraped-surface heat exchanger (SSHE). The rotating scraper blades of a SSHE continuously remove product from the heating surface while a connected pump moves the product through the length of the heating tube, thus creating a stable heat transfer rate

throughout the entire run without fouling (Rao & Hartel 2006). SSHEs are commonly used for both heating and cooling products, with applications in confectionary products, sterilization of particulate products, and manufacture of ice cream. No research has been published on the application of SSHEs with hummus, though manufacturers of the equipment, including Terlet and TetraPak, advertise that SSHEs can process hummus. The application of SSHEs with the hot-fill hold method could be very desirable for hummus, as the closed heating environment minimizes water loss due to vaporization and a connected piston pump would enable direct filling into the final package. Exact settings to establish temperature requirements for thermal processing of hummus have yet to be published, and such research would be critical to adoption in manufacturing settings.

1.4.3 Sterilization Values

When determining temperature requirements for processing shelf-stable products, the final product must meet the requirements of a commercially sterile product. According to 21 CFR 114.8E, commercially sterile foods must have undergone a thermal process that ensures the food is a) free of microorganisms capable of reproducing under normal storage conditions and b) free of viable pathogens (including spores). To quantify the microbial lethality of a heat process, multiple researchers have developed time-temperature data with the elimination (or log reduction) of reference microorganisms at reference temperatures (Pflug 1982). The time required at the reference temperature to achieve a degree of microbial kill is defined as the sterilization value, also called the lethality value or F value. From the F value at the reference time, the F value at any other temperature can also be calculated from the following equation:

$$\frac{F(T)}{F(T_{ref})} = 10^{(T_{ref}-T)/z}$$

Where T is the temperature of interest, T_{ref} is the reference temperature, and z is the z-value used with the reference temperature for the reference microorganism (Pflug 1982). This equation is extremely useful, as it allows the equivalent microbial kill time to be calculated for any temperature and provides evidence for the safety of a thermal process in food production.

With the hot-fill hold method when making shelf-stable products, the lethality conferred must be considered for the food packaging as well. Heat transfer from the food to the package is product-specific and depends on the container size and material type. Therefore, time and temperature data is typically collected when developing a thermal processing regime for a new type of product and package. Heat penetration data will be imperative for this project to determine the efficacy of a temperature treatment and will likely also elucidate the effects of hurdle combinations with the thermal process.

1.4.4 Importance of Vacuum

Obtaining a vacuum with hot-filled products is critical to establishing a hermetic seal on the finished product. Specifically, a hermetic seal ensures that the container is airtight and protects the food contents against the entry of microorganisms during and after processing, thereby maintaining the commercial sterility of the product (GMA-SEF 2007b). When a vacuum is present, it normally indicates that the hermetic seal is intact and helps consumers confirm that a product is not spoiled. The vacuum of a finished product can be measured with a hand-held vacuum gauge, with an adequate vacuum for acidified foods generally being greater than 6 inches mercury. However, it is suggested to be within the range of vacuum recommended by the container manufacturer (FDA 2009).

Several factors impact the formation of a good vacuum. An adequate amount of headspace is required to ensure that there is sufficient steam trapped after capping to create a

vacuum. While the correct headspace amount varies with product and process decisions, the general rule is no less than 6% headspace, with 8-10% headspace being the common recommendation (GMA-SEF 2007c). Product temperature at sealing also affects the vacuum as the product will contract upon cooling, with higher fill temperatures resulting in higher vacuum in the final package (GMA-SEF 2007c). Additionally, minimizing air in the product itself helps maintain the vacuum. If air is present in the product during sealing and then diffuses into the headspace, the vacuum will be lowered and might compromise the integrity of the hermetic seal.

Beyond helping establish a good seal, creating a vacuum also reduces the stress on the container, ensuring that its seams are not strained throughout processing and it does not burst during distribution. A vacuum creates a low-oxygen environment as well, which minimizes oxidation of fats or lipids and prevents discoloration of the product (GMA-SEF 2007b). If certain microorganisms are obligate aerobes, then the low-oxygen environment is also inhibitory to their growth in the product, should these microorganisms be present in the food product.

Given the importance of vacuum, it will be essential for the hummus processing parameters in this project to establish a good vacuum by reserving adequate headspace, treating the product at sufficiently high temperatures, and minimizing air incorporation into the hummus.

1.4.5 Finished Product Inspection by Incubation

With the production of shelf-stable products, it is imperative that only safe and stable products are distributed to consumers. For acidified foods, the specifications for pH and thermal treatment (temperature and time) must be met, and these parameters are typically detailed in the manufacturer's scheduled process or HACCP plan. However, when developing a new process on a new type of product, there are no pH or thermal treatment guidelines available. In such a case, finished product inspection protocol should be followed. This method is modeled off of the

USDA-FSIS incubation program outlined in 9 CFR 318.309 and 9 CFR 381.309 in which a select number of finished samples from each production batch must be incubated at 35°C ± 5°C for at least 10 days. The incubated samples are inspected daily for any abnormalities, which include bulging or swelling containers and product leakage from unopened containers. With acidified foods, such spoilage is usually due to mesophilic yeast, molds, and lactic acid bacteria, all of which are unacceptable from a quality standpoint (Denny & Corlett 1992). Should spoilage be observed in any samples from one batch, then that batch must be retained. If all containers from one batch appear normal after the incubation period, then the batch of product is considered fit to be shipped for consumption.

Until the process parameters are confirmed, product incubation and inspection is the best non-destructive method available to confirm the safety and quality of a shelf-stable product.

1.5 Objective of Study

Although there has been research on improving the safety and quality of hummus products, few have fully considered the holistic compilation of pH, acid addition, antimicrobials, and heat treatment. These preservative factors, called hurdles, can be combined in a scientifically based manner to increase their efficacy with preservation, a technique called hurdle technology (Leistner 2000). Hurdle technology is proposed to work by affecting the homeostasis, metabolic exhaustion, and stress reactions of the microorganisms in multiple ways at once (Leistner 2000). Thus, lower intensities of individual hurdles may be employed that still achieve the desired microbial stability and sensory quality of the food product. Although larger manufacturers in the U.S. have successfully combined thermal treatment of hummus with use of potassium sorbate or sodium benzoate to extend refrigerated shelf life to approximately 60 days, smaller producers do not have the capacity to thermally process hummus at such high

temperatures, highlighting a need to investigate alternative shelf life extension methods. Moreover, chemical preservatives carry a negative stigma, and natural antimicrobials could be better employed for the hummus industry.

Hurdle technology must consider the multiple effects of multiple treatments, and scientific basis is the soundest way to evaluate such effects. In several of the studies previously mentioned, pH differences of hummus samples were not controlled during antimicrobial trials (Alali et al. 2012, Al-Holy et al. 2006, Almualla et al. 2010, Amr and Yaseen 1994), and the pH values were not even reported for some hummus trials (Khiyami et al. 2011, Singh et al. 2001). Thus, the potential synergistic effects of the formulation or process parameters could not be determined, highlighting a need for further research. Moreover, the importance of maintaining organoleptic quality, particularly taste, while ensuring safety was not typically considered with these research projects, which is a major disadvantage of the current literature available.

The objective of this research project was to develop formulation and processing parameters to ensure the safety and optimal quality of hummus. The antimicrobial effects of two main acids, citric acid and GDL, were assessed in combination with acetic acid in hummus formulations. Citric acid and GDL were chosen as the main acids because the former is the primary acid currently used in hummus and the latter has less of an acidic taste, which could be advantageous for hummus products requiring low pH. Acetic acid was chosen for its antimicrobial effects previously discussed in Section 1.3.2 and for its commonality as a food ingredient within vinegar. These acids were employed to lower the hummus pH to 4.6 and 4.2 in two studies. In addition to the acids, four different antimicrobial preservatives were tested in the formulation: natamycin (natural microbial preservative), plant bitters (natural plant-based preservative), potassium sorbate (chemical preservative), and sodium benzoate (chemical

preservative). The hummus formulations were challenged with mold inoculation and stored at both accelerated and refrigeration temperatures to determine which acid-antimicrobial combination provided the best shelf life for hummus. In addition, consumers evaluated the acid-combination systems in hummus for flavor perception.

In the third and final study, the acid-antimicrobial combination determined to be most successful was trialed with three fill temperatures (76.7°C, 82.2°C, or 87.8°C) to produce a shelf-stable hummus at pH 4.2 utilizing the hot-fill hold method. The formulation was compared against a control with no antimicrobial and against potassium sorbate with sodium benzoate. All systems were challenged with mold inoculation. Compared to other processing options, the hot-fill hold method is the most accessible for small-scale producers, making it the ideal method to trial for a hurdle technology that could minimize energy costs and expensive equipment purchases. From the results of physical attribute analyses and microbial enumeration, the best temperature-formulation combination will be identified for the production of shelf-stable hummus.

The results of the study will be of particular relevance to small producers that do not have the same large-scale manufacturing capacity of larger companies. At the Food Venture Center, requests for assistance with or approval of new hummus products has continuously increased over the years, in which scheduled processes must be drafted that include formulation, critical control points, processing steps, and storage, distribution and selling conditions/restrictions (NECFE 2013). Thus, the scientific-based guidelines of formulation and processing developed from this research will be of great assistance in writing and approving scheduled processes for hummus while ensuring the safety of such products on the market.

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CHAPTER 2
EXTENDED SHELF LIFE OF REFRIGERATED HUMMUS WITH NATURAL ANTIMICROBIALS AND ACID-COMBINATION SYSTEMS

1. INTRODUCTION

Hummus is a traditional Middle-Eastern dip made from cooked chickpeas mashed with tahini (sesame paste) and typically flavored with lemon juice, salt, and garlic (Al-Holy et al. 2006). With the growing popularity of ethnic cuisines in the United States, hummus consumption has increased rapidly over the past few years, totaling $530 million in sales in 2012 and having increased 25% from 2010 (Kesmodel & Fletcher 2013). Consumers associate hummus with the health benefits of a Mediterranean diet (Browne 2011), being higher in protein and lower in fat than many other spreads or dips. However, hummus presents multiple food safety risks, being high in water activity (>0.98) and traditionally not heavily acidified (pH > 4.6). Also, the raw ingredients can introduce different microorganisms and it is not traditionally heat-treated (Yamani and Al-Dababseh 1994). A multistate outbreak of *Salmonella* serotype Bovismorbificans in 2011 was ultimately linked to hummus and tahini (Mortality and Morbidity Weekly Report 2012), highlighting the need for scientific guidelines in formulation and processing to minimize food safety risks and improve quality.

Acidified foods are required to have a pH of ≤ 4.6 to inhibit the growth of *Clostridium botulinum* (FDA 2014), and addition of acid also helps mitigate growth of other pathogens. Citric acid, the predominant acid in lemon juice, has been shown to not have as strong of an antimicrobial effect against *Shigella* species as lactic or acetic acids (In et al. 2013). Acetic acid has also demonstrated strong bactericidal effects against *Listeria* and *E. coli* O157:H7 (Kreske et al. 2008) and against mold species (Lind et al. 2005). This has led to multiple scientific guidelines to use acetic acid in food formulation or processing to ensure safety and quality

(Sullivan et al. 2013, Khurana et al. 2006), which could be applied to hummus. Glucono-delta-lactone (GDL) is a slow-release acidulant with a mild taste (Pszczola 2007). Its demonstrated antimicrobial effects against *Listeria monocytogenes* in frankfurters and bologna (Samelis et al. 2002, Barmpalia et al. 2005) may be advantageous in hummus as well.

Mold is a quality concern with hummus, and most commercial hummus manufacturers employ chemical preservatives (potassium sorbate or sodium benzoate) to extend refrigerated shelf life to 60 days. However, there is growing consumer sentiment against chemical additions to food products, with high demand for natural ingredients while still having a long shelf life (Zink 1997). Natamycin, an antimycotic polyene macrolide produced from the fermentation of dextrose by *Streptomyces natalensis*, has been used as a natural preservative in certain foods and beverages to prevent spoilage from growth of yeasts or molds but not from growth of bacteria. Given its low solubility in water (40 mg/ml), natamycin is more commonly used as a surface treatment but has been reported as an effective preservative in biofilms and juices (Ture et al. 2011, Hondrodimou et al. 2011, Siricururatana et al. 2013). Certain plant extracts also exhibit antibacterial and antifungal activity, and these compounds can be considered natural preservative ingredients as well (Davidson et al. 2013).

With growing concerns about chemical preservatives, natural acid systems and natural antimicrobials could be a desirable alternative. The effectiveness of four acid systems (citric, citric plus acetic, GDL, GDL plus acetic) were studied in combination with two natural preservatives, natamycin and plant bitters, against the chemical preservative potassium sorbate in refrigerated hummus at pH 4.6 under accelerated storage conditions. The acid-antimicrobial combination that was determined to confer the best microbial stability and product quality was evaluated at refrigerated temperatures in hummus at pH 4.6 and 4.2 to assess maximum shelf life.

2. MATERIALS AND METHODS

2.1 Phase I – Accelerated Refrigerated Storage

2.1.1 Materials for Hummus Preparation

Retorted chickpeas (garbanzo beans) were obtained from the Goya Foods manufacturing facility in Angola, NY. Tahini, olive oil, and 5% acetic acid (white vinegar) were purchased from the local supermarket (Geneva, NY). Sodium chloride was supplied by Fisher Scientific (Pittsburgh, PA) and citric acid was supplied by J.T. Baker Chemicals (Center Valley, PA). Glucono-delta-lactone (GDL) and potassium sorbate were supplied by Acros Organics (New Jersey). Natamax®, 50% natamycin, was supplied by DuPont Chemicals (New Century, KS). Plant bitters were supplied by SIMPLE iDEAS Inc. (Surprise, AZ). All reagent solutions were prepared with distilled water.

2.1.1.1 Natamax® by DuPont Chemicals

Dupont Chemicals (formerly Danisco) produces Natamax® for use in food formulation as a preservative. Natamax® is prepared as a powder in which natamycin is blended with lactose 50-50% by weight. The natamycin is food grade and meets the regulations and specifications of the FAO/WHO, the EU, and the U.S. Code of Regulations. The product is certified kosher dairy and certified Halal. The only declared major allergen is dairy.

2.1.1.2 Plant Bitters by SIMPLE iDEAS Inc.

SIMPLE iDEAS Inc. produces plant bitters for use in food formulation as a natural flavor. The plant bitters are a viscous yellow liquid, with its active ingredient being noted as plant extracts but no specific plants listed. According to manufacturer documentation, the method of extraction for the plant extracts is glycerine heat extraction. Inactive ingredients are glycerin, water, grain alcohol, and ascorbic acid. The alcohol content is 9.1% and there are no major allergens.

2.1.2 Hummus Preparation – Phase I

Hummus batches of 600g were prepared as follows, with formulation percentages listed in **Table 2.1**. Ingredient ratios were optimized in preliminary research completed by Summer Scholar Sara Spoede to achieve viscosity that was comparable to commercial hummus (data not shown). Chickpeas were drained from the cooking liquid and rinsed in cold water prior to being pulsed into a smooth paste with a Robot Coupe R6VN (Ridgeland, MS) food processor. The chickpea paste was transferred to a smaller Robot Coupe R302V, and tahini, olive oil, and sodium chloride were added to the food processor and pulsed until combined. This base hummus was then acidified by addition of the appropriate level of corresponding acid, detailed in **Table 2.2**, to achieve the targeted pH of 4.6. Citric acid was delivered as a 50% citric acid solution, and acetic acid was delivered as white vinegar (5% acidity). Subsequently, one of four antimicrobial agents was added: 40 ppm Natamax® (equivalent to 20 ppm natamycin), 1000 ppm potassium sorbate, 500 ppm plant bitters, or 1000 ppm plant bitters. The Natamax® powder was mixed with the portion of sodium chloride prior to addition to adequately disperse the powder. No antimicrobial agent was added to one batch per acid system as a control. Distilled water was added to reach 100% of formulation. Batches were prepared in triplicate, resulting in three replicates per acid-antimicrobial combination. Hummus batches were separated into 100-g samples in polylactic acid (PLA) containers with snap-on lids. Samples were refrigerated overnight (4°C) prior to mold inoculation to achieve pH equilibration.

Table 2.1 Base hummus formulation

Ingredient	Percentage (% w/w)
Chickpeas	74.0
Tahini	4.0
Olive oil	10.0
Table Salt (NaCl)	0.5
Acid addition	0.365-0.81
Antimicrobial addition	0.004-0.1
Water	~11 (to total 100%)

Table 2.2 Acid additions to achieve targeted pH value of 4.6 in hummus samples

Acid System	Citric acid (% w/w)	Acetic acid (% w/w)	GDL (% w/w)
Citric only	0.365	-	-
Citric + Acetic	0.31	0.061	-
GDL only	-	-	0.81
GDL + Acetic	-	0.125	0.63

2.1.3. Mold Isolation and Inoculation

Mold was isolated from spoiled commercial hummus samples by plating on potato dextrose agar (PDA) acidified to pH 3.5 with 10% tartaric acid and was identified as *Penicillium* by microscopic observation. *Penicillium* is a common spoilage mold in refrigerated foods and is able to grow at low temperatures (Pitt 2006), making it an ideal organism with which to challenge the hurdle system in hummus. To prepare the inoculum, mycelia was gently scraped off the agar surface with a sterile scalpel and placed in a sterile plastic tube with 25 mL Millipore water. Ten-fold serial dilutions to 10^{-6} of the initial suspension were prepared in peptone water. The 10^{-3} dilution was confirmed to contain approximately 1.36 x 10^{4} CFU/mL by plating 1 mL on acidified PDA, incubating at 30°C, and counting colonies after 72 h of incubation.

For the shelf-life challenge study, each 100-g hummus sample was individually inoculated with 0.750 mL of the 10^{-3} inoculum dilution to achieve approximately 100 CFU per gram of hummus, and the sample was stirred with a sterile glass rod to ensure uniform distribution. The inoculated sample cups were then recapped and placed in 15°C incubator for accelerated storage over five weeks.

2.1.4 Storage of Samples for 5-week accelerated study

Hummus samples were stored for 5 weeks and were inspected daily for visible mold spoilage. Samples were chosen to be stored at 15°C to accelerate the microbial activities and to predict what the shelf life will be at 5°C by applying the Q_{10} temperature coefficient. The Q_{10} temperature coefficient is defined as the change in the reaction rate constant, K, for a change in

temperature of 10°C (Pflug 1982). As a food product's shelf life is dependent on the rate of spoilage reactions from microorganisms, it is directly related to Q_{10} as well:

$$Q_{10} = \frac{rate\ at\ T + 10℃}{rate\ at\ T} = \frac{shelf\ life\ at\ T}{shelf\ life\ at\ T + 10℃}$$

Where T = temperature (Fu and Labuza 1997). For many chemical and biological reactions, the general Q_{10} value is estimated to be 2. Therefore, the shelf life at 5°C is predicted to be double the shelf life observed at 15°C from the accelerated study.

2.1.5 Microbial Sampling

One hummus sample per replicate was tested once each week for five weeks to measure the microbial load. If a sample had visible mold or yeast growth on the surface, then the sample was not tested and noted as "spoiled" with an assumed microbial count $\geq 10^6$ CFU/g hummus. To perform the microbial sampling, 25 g of hummus was weighed into a stomacher bag and diluted with 225 g of 0.1% peptone water to prepare a 1:10 dilution. The diluted sample was then blended in a Stomacher® 400 (Seward USA, Davie FL) for two minutes at 230 RPM. Serial dilutions were made in sterile 0.1% peptone water, and 1-ml samples of the various dilutions were pour-plated in duplicate on acidified PDA. The plates were incubated for 72 h at 30°C, and plates of the appropriate dilutions (25 to 250 colonies) were chosen for colony enumeration of yeasts and mold.

Total plate count was performed on the starting week (Week 0) and the final week (Week 5) by following the dilution method previously described and pour-plating on standard plate count agar (PCA). Plates were incubated for 72 h at 30°C, and plates of the appropriate dilutions (25 to 250 colonies) were chosen for colony enumeration. Lactic acid bacteria were enumerated on Week 3 or Week 4 by plating on deMan Rogosa Sharpes (MRS) agar. MRS plates were incubated at 30°C and enumerated after 48 h.

2.1.6 pH Measurement

The pH values of the samples were measured weekly to monitor changes in acid production by yeasts, molds, or bacteria, if any. All pH measurements were made with an Orion 8172BNWP ROSS Sure-Flow pH Electrode (ThermoScientific, Hanover Park, IL) connected to an Orion 3 Star pH meter (ThermoScientific, Hanover Park, IL). Measurements were taken once the samples reached ambient temperature (25°C).

2.1.7 HPLC Analysis of Natamax® and Plant Bitters

To confirm the concentration of natamycin in Natamax®, the Natamax® sample was analyzed with high-performance liquid chromatography (HPLC). To prepare a standard solution of natamycin, an analytical standard of natamycin (Vetranal® from Sigma-Aldrich, St. Louis MO) was dissolved in an aqueous solution of 10% acetonitrile and 0.1% phosphoric acid. Standard solutions of 1, 5, 10, 20, 50, and 100 ppm natamycin were prepared. Ten milligrams of Natamax® was dissolved in 100 mL of the same aqueous solution as above to create a 100 ppm Natamax® solution for analysis. Two additional Natamax® solutions at 20 ppm and 50 ppm were prepared in the same manner. HPLC analysis was completed following the protocol as described by Manns et al. (2012). The specifications of this protocol are listed in **Table 2.3**. The method was completed using an Agilent 1260 Infinity series HPLC (Agilent Technologies, Santa Clara, CA) and a C_{18} column (100 mm x 4.6 mm) packed with 2.6 μm particles with a 100Å pore size. For this analysis, the wavelengths monitored were 320 nm and 305 nm, based on recommendations from the natamycin standard supplier.

The plant bitters sample was also analyzed by HPLC to identify the primary compounds present, as no specifics of plant origin were given. The plant bitters sample was diluted in distilled-deionized water at four ratios: 50%, 10%, 1%, and 0.1%. HPLC analysis was

completed following the protocol as described by Manns et al. (2012), with the same specifications listed in **Table 2.3**. Wavelengths monitored included 280, 320, 360, and 520 nm.

Table 2.3 HPLC conditions used for the analysis of Natamax and plant bitters following the protocol of Manns et al. (2012)

Parameter	Time (min)	% Mobile Phase B
Elution gradient		
	0	5
	2	5
	6	12.5
	8	15
	13	80
	14	100
	16	100
	17	5
Injection vol. (μL)	20	
Flow rate (mL/min)	2	
Mobile phase A	H_2O:H_3PO_4 (99.5:0.5)	
Mobile phase B	ACN: H_2O:H_3PO_4 (50:49.5:0.5)	
Starting run pressure (bar)	250	
Minimum run pressure (bar)	235	
Maximum run pressure (bar)	265	
Column temp. (°C)	45	
Run time (min)	17	
Post time (min)	3	

2.1.8 Statistical Analysis

Microbial counts were analyzed within acid-combination groups by one-way analysis of variance (ANOVA), and total plate counts were analyzed by the Student t-test for significant changes during storage. The pH values were analyzed by logistic regression within each system treatment for changes during the 5-week storage period. All statistical analysis was completed using JMP 11.0 Statistical Software (Cary, NC). Statistical significance was defined at $p < 0.05$.

2.2 Phase II – Real-time refrigerated storage

2.2.1 Materials for Hummus Preparation

The same materials listed in Phase I were also used for hummus preparation in Phase II, excluding GDL and plant bitters. In addition, sodium benzoate was procured from Afla Aesar (Heysham, England). All reagent solutions were prepared with distilled water.

2.2.2 Hummus Preparation – Phase II

Hummus batches of 850g were prepared in the manner previously described for Phase I, with the same base formulation (**Table 2.1**). Following completion of the base hummus, the hummus was then acidified by addition of the appropriate level of citric and acetic acids, detailed in **Table 2.4**, to achieve the target pH of 4.60 or 4.20. Higher amounts of acid were added for the potassium sorbate and sodium benzoate batches because of the basic nature of those ingredients. Subsequently, one of three antimicrobial agents was added: 40 ppm Natamax®, 1000 ppm potassium sorbate, or 1000 ppm sodium benzoate. No antimicrobial agent was used in one batch as a control. Batches were prepared in triplicate, resulting in three replicates per antimicrobial treatment. Hummus batches were separated into 60-g samples in PLA containers with snap-on lids. All hummus batches were refrigerated overnight (4°C) prior to mold inoculation to achieve pH equilibration.

Table 2.4 Acid additions (% w/w) to achieve targeted pH in hummus samples

Acid Type	Control pH 4.6	Control pH 4.2	Natamax pH 4.6	Natamax pH 4.2	Potassium sorbate pH 4.6	Potassium sorbate pH 4.2	Sodium benzoate pH 4.6	Sodium benzoate pH 4.2
Citric	0.31%	0.57%	0.31%	0.57%	0.34%	0.62%	0.38%	0.66%
Acetic	0.061%	0.114%	0.061%	0.114%	0.061%	0.114%	0.061%	0.114%

2.2.3 Mold Inoculation

The same mold inoculum of *Penicillium* was used as previously described for Phase I. For the shelf-life challenge study, each 58-g hummus sample was individually inoculated with 0.435 mL of the 10^{-3} dilution of mold inoculum to achieve approximately 100 CFU per gram of hummus and stirred with a sterile glass rod to ensure uniform distribution. The inoculated sample cups were then recapped and placed in a 5°C refrigerator for storage for 26 weeks.

2.2.4 Microbial Sampling

One sample per replicate was tested biweekly for 26 weeks to measure the microbial load. Yeasts and molds were enumerated on acidified PDA and total plate counts were enumerated on

PCA at each sampling point. If a sample had visible mold or yeast growth on the surface, then the sample was not tested and noted as "spoiled" with an estimated microbial count $\geq 10^6$ CFU/g hummus. Sampling, plating, and enumeration methods were followed as previously. Lactic acid bacteria were enumerated once every four weeks, starting on Week 4 for pH 4.2 hummus and on Week 6 for pH 4.6 hummus by plating on MRS agar as previously described.

2.2.5 pH Measurement

The pH values of the samples were measured biweekly as previously described to monitor changes in acid production by yeasts, molds, or bacteria, if any.

2.2.6 Sensory Evaluation

A triangle discrimination test was conducted in the Cornell Sensory Testing Facility in Geneva, NY to determine if consumers can perceive a flavor difference between citric only and citric plus acetic hummus formulations. The sensory test was reviewed and approved by the Institutional Review Board of Cornell University. Panelists were recruited from the New York State Agricultural Experiment Station via email, were of 18 years of age or older, and were asked not to participate if they had a sesame allergy. A total of 42 panelists completed the discrimination test. A triangle test was chosen because the task is simple for untrained panelists to complete and the differing sensory aspect does not have to be specified (Lawless and Heymann 2010). Two sets of triads were presented to panelists, one of hummus samples at pH 4.6 and one of samples at pH 4.2. In each triad, two samples were of the same acid system (citric or citric-acetic) and one sample was the alternate acid system. Samples were prepared following the hummus preparation methods previously described, and formulations are listed in **Table 2.5**. The hummus was packaged as 40-g samples in PLA cups with lids and refrigerated at 5°C until immediately prior to the taste test.

Table 2.5 Hummus formulations for sensory evaluation study made with A) citric acid, pH 4.6; B) citric acid, pH 4.2; C) citric + acetic acids; pH 4.6; & D) citric + acetic acids, pH 4.2

A. Citric acid, pH 4.6

Ingredient	Percentage (% w/w)
Chickpeas	74.00
Tahini	4.00
Olive oil	10.00
Table Salt (NaCl)	0.50
Citric Acid	0.37
Acetic Acid	0.00
Water	11.10

B. Citric acid, pH 4.2

Ingredient	Percentage (% w/w)
Chickpeas	74.00
Tahini	4.00
Olive oil	10.00
Table Salt (NaCl)	0.50
Citric Acid	0.62
Acetic Acid	0.00
Water	11.10

C. Citric + Acetic acids, pH 4.6

Ingredient	Percentage (% w/w)
Chickpeas	74.00
Tahini	4.00
Olive oil	10.00
Table Salt (NaCl)	0.50
Citric Acid	0.32
Acetic Acid	0.064
Water	11.10

D. Citric + Acetic acids, pH 4.2

Ingredient	Percentage (% w/w)
Chickpeas	74.00
Tahini	4.00
Olive oil	10.00
Table Salt (NaCl)	0.50
Citric Acid	0.53
Acetic Acid	0.106
Water	11.10

Panelists were seated at partitioned booths and presented with three samples from the first set simultaneously. To minimize visual comparison, the sensory booths were lit with red light. The written instructions on the ballot stated: "Taste each of the three samples in the order presented from left to right. Indicate which sample is different by circling the code of the different sample on the score sheet below. Be sure to rinse your mouth with water prior to beginning and between each sample." A sample ballot is provided in Appendix A. Panelists tasted the samples using plastic spoons and recorded responses on the paper ballots. After finishing the first triad set, the second triad was provided. Sample presentation was randomized within triads and counterbalanced among the panelists. The order of set presentation was in increasing acidity (i.e. Set 1: pH 4.6 and then Set 2: pH 4.2) for all panelists to minimize carryover effects from acidity. The number of panelists that correctly identified the odd sample from each hummus set was recorded.

2.2.7 Statistical Analysis

Microbial counts were analyzed among antimicrobial treatments by one-way analysis of variance (ANOVA). Differences in total plate counts and shelf life between the pH 4.6 and pH 4.2 batches were analyzed by the Student t-test. All statistical analysis was completed using JMP 11.0 Statistical Software (Cary, NC). Statistical significance was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phase I – Accelerated Refrigerated Storage

3.1.1 Microbial Enumeration – Yeasts and Mold

The results of yeast and mold (YM) enumeration from the hummus samples during five weeks of 15°C storage are presented in **Figure 2.1**. Hummus samples treated with natamycin and acidified with citric-acetic acids had the lowest YM counts at Week 5 compared to all other antimicrobial treatments in this acid system ($p < 0.01$). The YM counts of the natamycin citric-acetic samples were also stable over all five weeks and even significantly decreased by Week 3 from the initial counts ($p < 0.05$). This decrease in YM counts may indicate that natamycin has a fungicidal effect on *Penicillium* when used with citric and acetic acids. In addition, there was no visible mold spoilage on any natamycin citric-acetic samples through Week 5. All other treatments in the citric-acetic acid system had at least one sample spoiled by Week 3 (**Table 2.6**).

In contrast, control samples and both plant bitter-treated samples in the citric-acetic system had rapidly increasing YM counts. Citric-acetic samples treated with potassium sorbate had initially stable YM counts, but counts had increased by Week 5 and there was visible spoilage while natamycin samples remained spoilage-free. Thus, the natamycin treatment was the most successful for inhibiting mold growth in the citric-acetic acid system.

Table 2.6 Spoilage by mold in hummus samples during storage at 15°C

		Number of samples (out of 3) spoiled by mold at:					
Acid system	Antimicrobial treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Citric	Control	0	1	2	3	2	3
	Natamycin	0	0	0	0	0	0
	Potassium Sorbate	0	0	1	1	2	2
	Plant Bitters 500	0	2	1	1	1	2
	Plant Bitters 1000	0	0	0	1	2	3
Citric + Acetic	Control	0	3	2	2	2	3
	Natamycin	0	0	0	0	0	0
	Potassium Sorbate	0	0	0	2	2	2
	Plant Bitters 500	0	2	0	2	2	2
	Plant Bitters 1000	0	0	1	2	1	2
Glucono-delta-lactone (GDL)	Control	0	0	0	1	2	2
	Natamycin	0	0	0	0	0	1
	Potassium Sorbate	0	0	0	1	3	2
	Plant Bitters 500	0	0	1	0	1	2
	Plant Bitters 1000	0	1	2	3	2	2
GDL + Acetic	Control	0	0	0	1	1	2
	Natamycin	0	0	1	2	1	2
	Potassium Sorbate	0	0	1	2	2	3
	Plant Bitters 500	0	1	0	0	1	2
	Plant Bitters 1000	0	0	1	1	2	2

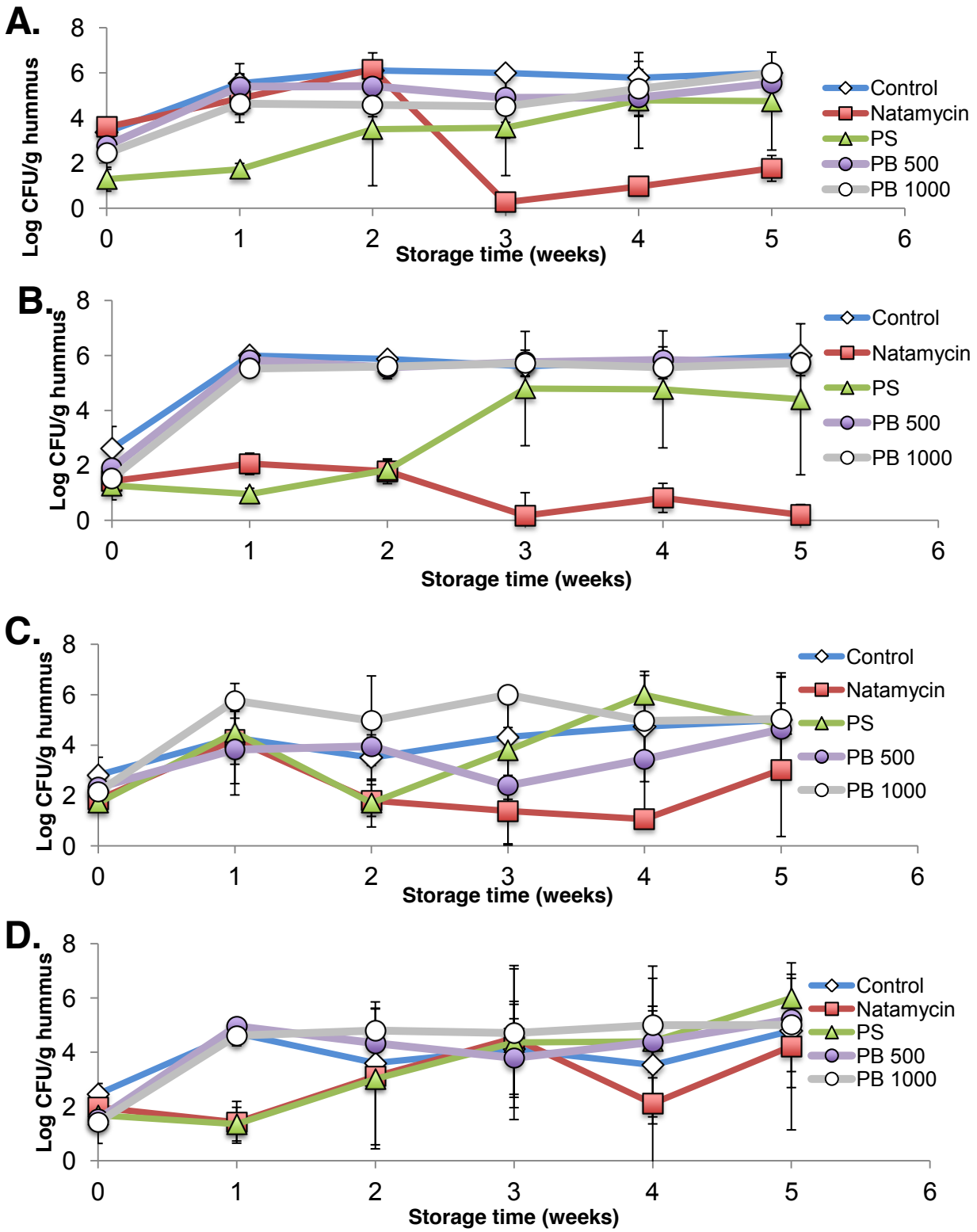

Figure 2.1 Changes in yeast and mold counts over storage period at 15°C for A) citric acid B) citric-acetic acid C) Glucono-delta-lactone (GDL), & D) GDL-acetic hummus [*in legend, PS = Potassium sorbate, PB 500 = plant bitters 500 ppm, PB 1000 = plant bitters 1000 ppm]*

Hummus samples acidified with citric acid and treated with natamycin had significantly lower ($p < 0.05$) YM counts at Week 5 compared to all other microbial treatments in the citric acid system. However, YM counts of the natamycin-citric samples were initially very high and then decreased, only to increase again by Week 5. This antimicrobial-acid system showed more instability than the natamycin-treated citric-acetic samples. No spoilage was observed in any natamycin samples in the citric acid system, while all other antimicrobial treatments in this acid system had at least one sample spoiled by Week 2 (**Table 2.6**). The YM counts of control and both plant bitters treatments did not differ significantly from each other over the five weeks ($p > 0.05$). Mold spoilage was observed in potassium sorbate samples during Week 2. Thus, the natamycin treatment was the most successful for inhibiting mold growth in the citric acid system.

Hummus samples acidified with GDL or GDL and acetic acid had no significant difference in YM counts among the antimicrobial treatments at Week 5 ($p > 0.05$). Samples from all antimicrobial treatments in these acid sytems had visible *Penicillium* spoilage by Week 3. Overall, no antimicrobial treatment performed better than the control in controlling yeast and mold growth with the GDL or GDL-acetic acid systems.

3.1.2 Microbial Enumeration – Lactic Acid Bacteria

Figure 2.2 displays the counts of lactic acid bacteria (LAB) from the hummus samples at the midpoint of the study. Grouping by acid-combination type, the LAB counts of the citric-acetic hummus samples were significantly lower ($p < 0.05$) than LAB counts of other acid-combination hummus samples. This result indicates that the combination of citric and acetic acids best inhibits LAB growth in hummus among the acid systems tested.

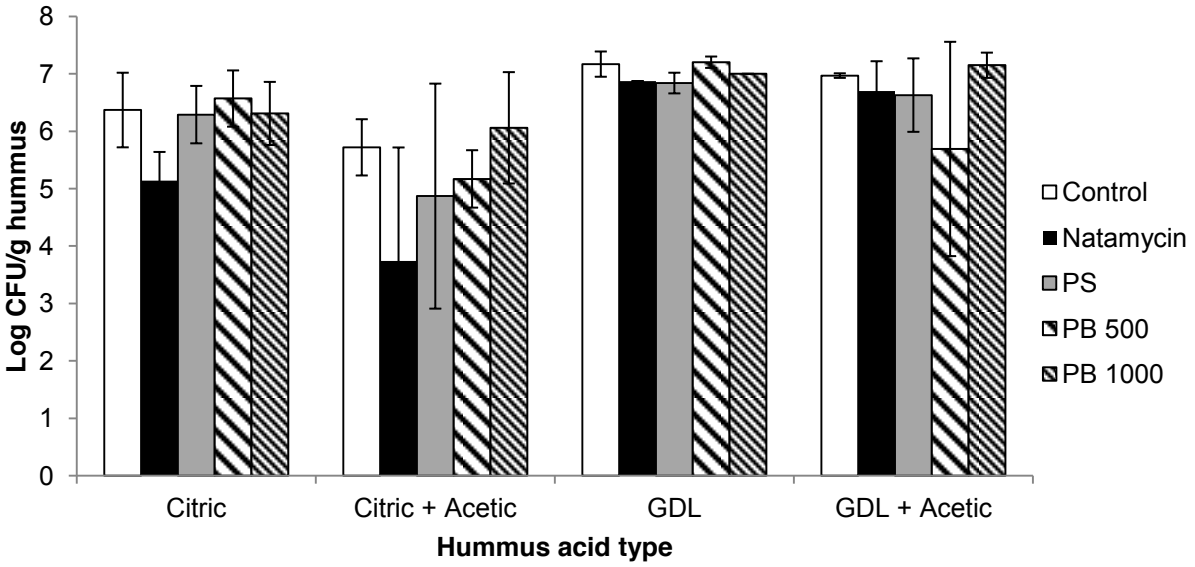

Figure 2.2 Lactic acid bacteria counts at Week 3 or 4 in pH 4.6 hummus stored at 15°C

3.1.3. Microbial Enumeration – Total Plate Count

The results of total plate counts (TPC) from the hummus samples at Week 0 and Week 5 of 15°C storage are displayed in **Figure 2.3**. All samples had counts greater than or very close to the spoilage limit (>6 log CFU/g) by Week 5 except for the citric-acetic hummus treated with natamycin. The citric-acetic natamycin samples had significantly lower TPC than all other samples ($p < 0.01$) at Week 5.

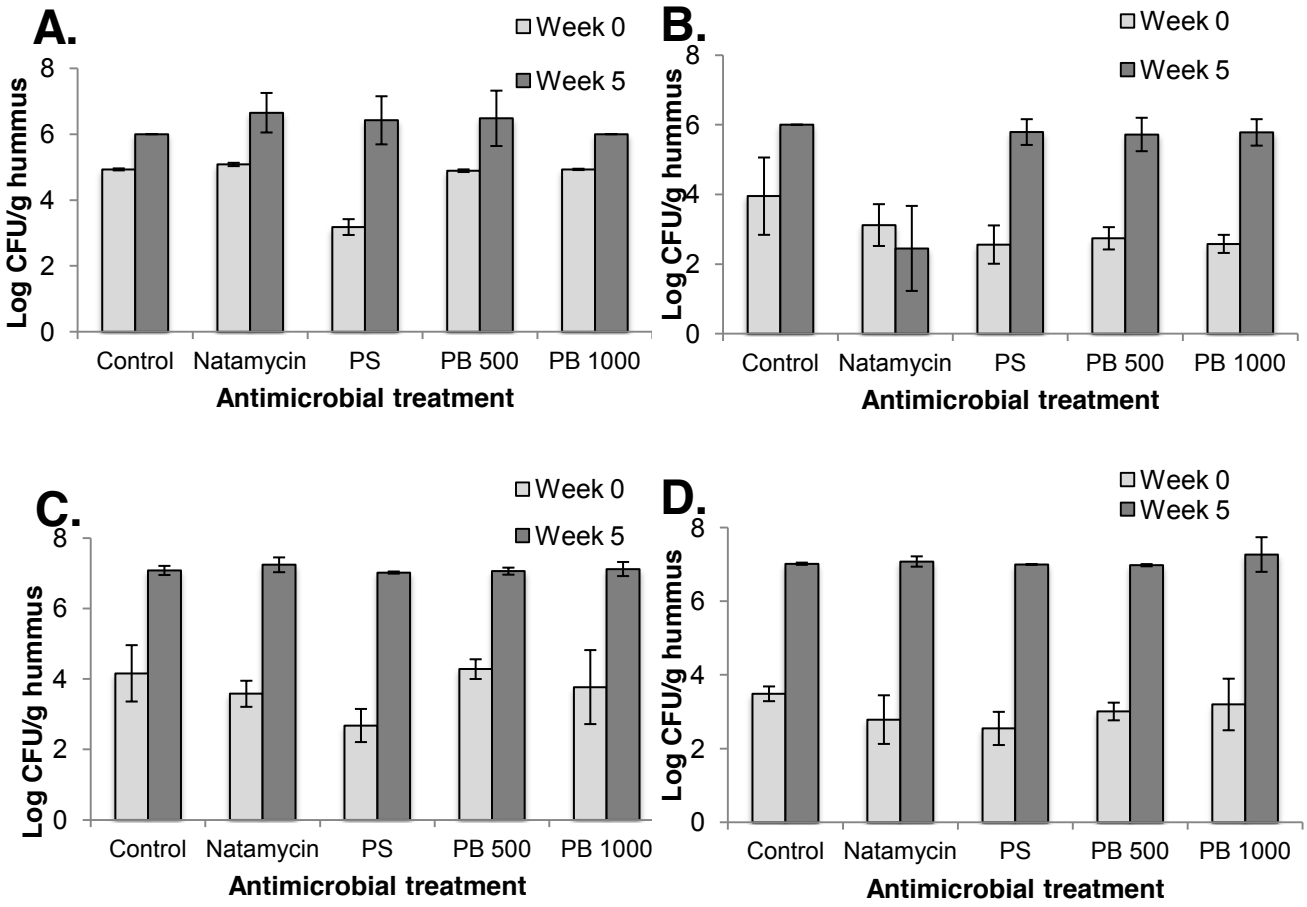

Figure 2.3 Changes in total plate counts over storage period at 15°C for A) citric acid B) citric-acetic acid C) Glucono-delta-lactone (GDL), & D) GDL-acetic hummus [*in legend, PS = Potassium sorbate, PB 500 = plant bitters 500 ppm, PB 1000 = plant bitters 1000 ppm]*

Figure 2.4 compares initial TPC with final TPC of hummus samples treated with natamycin across all four acid systems. While samples from the citric, GDL, and GDL-acetic acid systems had a statistically significant increase in TPC ($p < 0.05$) during storage at 15°C, there was no significant change ($p > 0.05$) in TPC of citric-acetic samples over the five weeks. These results further demonstrate that the natamycin-treated citric-acetic formulation best controls microbial growth for extended shelf life.

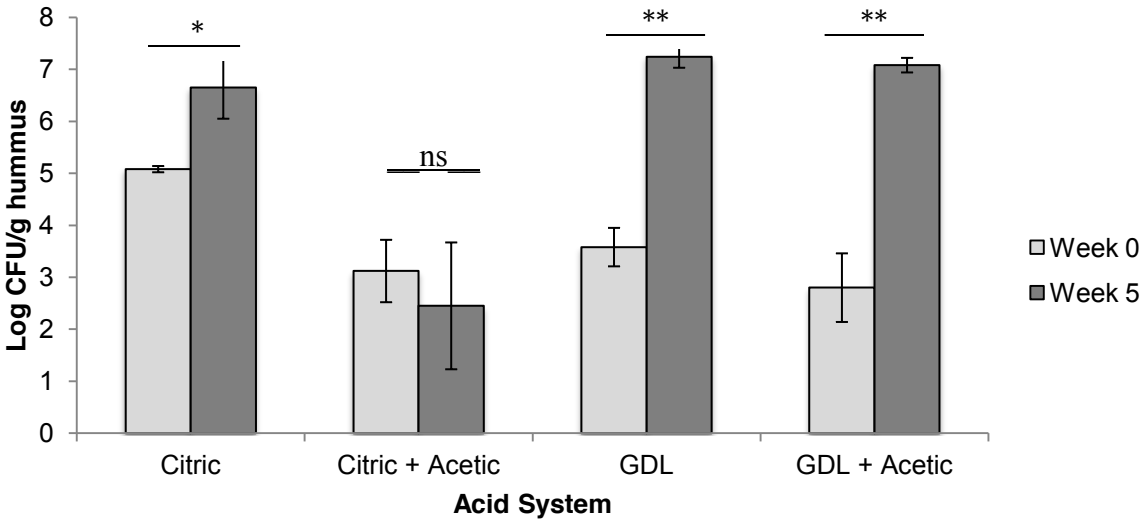

Figure 2.4 Changes in total plate counts in natamycin-treated hummus of differing acid systems over storage period at 15°C
*(GDL = Glucono-delta-lactone, * = $p < 0.05$, ** = $p < 0.01$, ns = no significant difference)*

3.1.4 Changes in pH

Figure 2.5 displays the changes in pH of the hummus samples over 5 weeks of storage at 15°C. There were no statistically significant changes in pH ($p > 0.05$) with any citric-acetic hummus samples. In contrast, for GDL hummus, all samples had statistically significant decreases ($p < 0.05$) in pH values, and for GDL-acetic hummus, all samples except those treated with potassium sorbate significantly decreased ($p < 0.01$) in pH. Results were mixed with citric hummus: control and natamycin samples had no significant pH change ($p > 0.05$) while the other treatments did have a statistically significant decrease in pH ($p < 0.01$).

The high LAB counts in citric, GDL, and GDL acetic hummus samples likely caused the observed decrease in pH with the production of lactic acid. As citric-acetic hummus had the lowest LAB counts, it is logical that there was no significant pH change in those hummus samples. It was concluded that the citric-acetic acid combination was the most effective in controlling microbial growth that resulted in minimal pH changes.

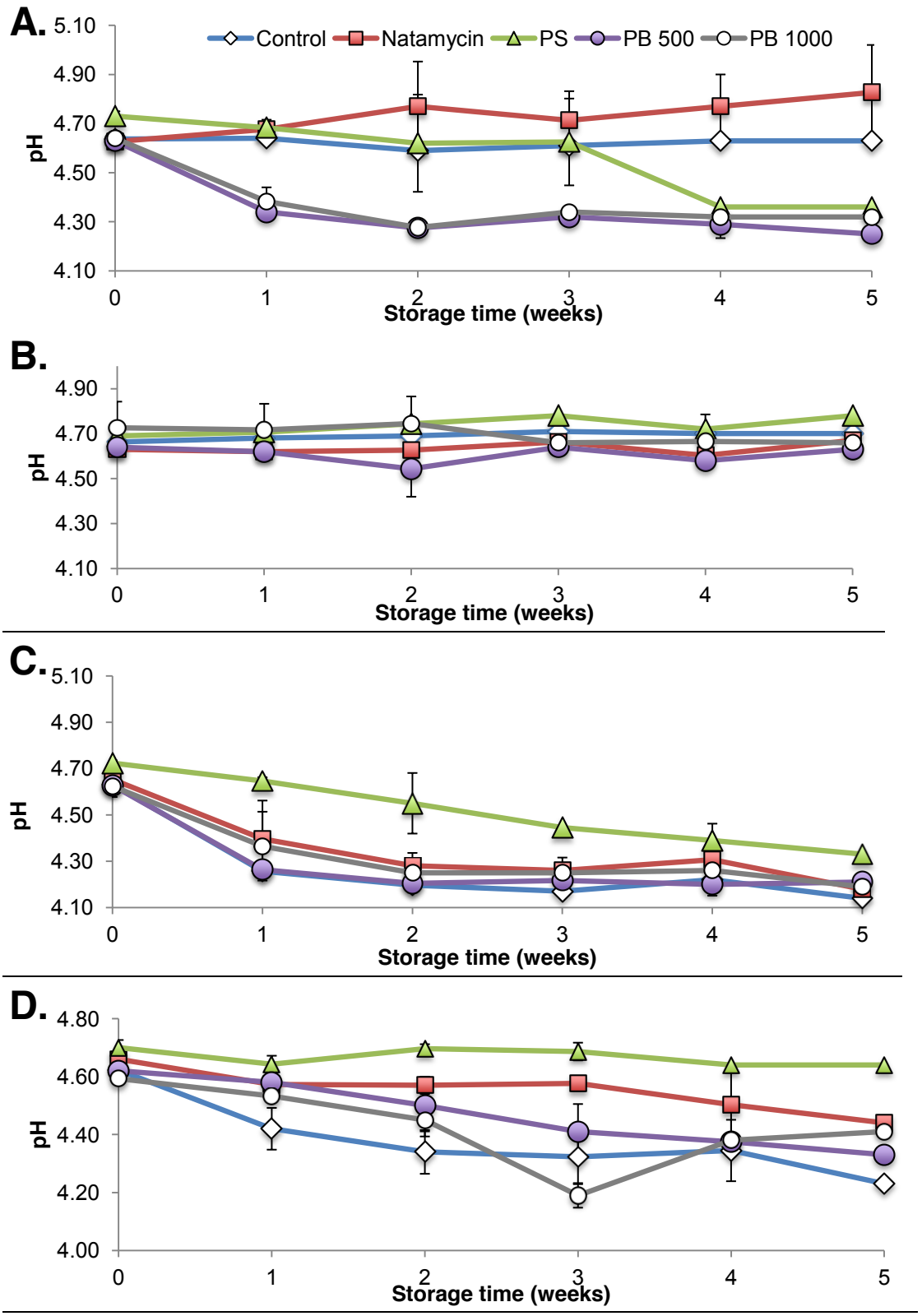

Figure 2.5 Changes in pH over storage period at 15°C for A) citric acid B) citric-acetic acid C) Glucono-delta-lactone (GDL), & D) GDL-acetic hummus [*in legend, PS = Potassium sorbate, PB 500 = plant bitters 500 ppm, PB 1000 = plant bitters 1000 ppm]*

3.1.5. Prediction of Shelf Life

Applying the criterion that TPC > 10^6 CFU/g exceeds consumer quality expectations for shelf life, citric-acetic hummus treated with natamycin was the only combination to pass shelf life at Week 5 at 15°C. With the Q_{10} value of 2 as previously discussed, a shelf life of five weeks at 15°C is estimated to be ten weeks of shelf life at 5°C. As no spoilage was observed at five weeks, it is possible that the shelf of natamycin-treated citric-acetic hummus could be longer than ten weeks at refrigerated temperatures.

Although the citric hummus samples treated with natamycin had no visible mold spoilage by Week 5, the TPC above 10^6 CFU/g indicates that its shelf life is less than five weeks. Visible spoilage of GDL and GDL-acetic natamycin-treated samples and the high TPC also indicates a shelf life less than five weeks at 15°C. Therefore, natamycin extends shelf life longer when used in combination with citric and acetic acids compared to the other acid systems.

Visible mold spoilage was observed for potassium sorbate-treated hummus by Week 2 or 3 for all acid systems, estimating a maximum of six weeks of shelf life at 5°C. Samples treated with plant bitters, either at 500 or 1000 ppm, had rapid mold spoilage similar to control samples without antimicrobials. Thus, plant bitters did not increase the shelf life of hummus regardless of acid system. GDL and GDL-acetic control samples had mold spoilage later in the study than citric and citric-acetic control samples, which may have been due to the byproducts produced by LAB, as lactic acid and other metabolic byproducts have demonstrated antifungal activity (Lind et al. 2005, Rouse et al. 2008). However, LAB growth is undesirable in hummus and TPC counts exceeded 10^6 CFU/g in all hummus samples, regardless of antimicrobial treatment, by Week 3. Thus, the GDL and GDL-acetic acid systems were not effective in controlling microbial growth to help extend hummus shelf life.

3.1.6. Natamycin Quantification in Natamax®

From HPLC analysis, the Natamax® solutions of 20 ppm, 50 ppm, and 100 ppm were determined to be 57.4%, 56.6% and 58.2% natamycin, respectively. Given lot-to-lot variation, these results confirm the manufacturer's guarantee that Natamax® is >50% natamycin. For the continuation of the study, Natamax® is assumed to be 50% natamycin.

3.1.7. Phenolic Composition of Plant Bitters

With the HPLC analysis of plant bitters, the most prominent peak was observed at 11.1 minutes at 280 nm, accounting for 75% of UV-detectable signal. Based on the elution time and the wavelength, it was hypothesized that the compound accounting for this peak was naringin. The prominent peak was overlaid with a naringin standard molecular absorption curve, and the curves were identical, confirming that the plant bitters compound was naringin. Using the naringin standard curve and absorption values of the 1% plant bitters solution, the plant bitters sample was determined to be 1.05% naringin.

Naringin is a bitter flavonoid commonly found in citrus fruits, particularly grapefruit. The compound has demonstrated inhibitory effects on the growth of periodontal pathogens and aerobic bacteria and yeasts commonly found in the oral cavity (Tsui et al. 2008). Earlier studies also observed bacteriostatic, fungistatic, and bactericidal effects from naringin (Cvetnic et al. 2004, Cushnie and Lamb 2005). However, the previous studies were completed *in vitro* with broth media, and the same antimicrobial effects from the plant bitters may not be observed in a complex food matrix. The failure of the plant bitters to extend hummus shelf life suggests that the active compound is not effective in a high-protein, high-fat environment. The plant bitters did inhibit mold growth in fruit jams at 55° Brix (Churey 2012, personal communication), so the antimicrobial properties may be best effective in low pH, low-fat water activity-controlled foods.

3.1.8. Conclusions from Phase I and Next Steps for Phase II

Among the acid systems and antimicrobial combinations tested, the citric-acetic hummus treated with natamycin had the longest shelf life based on low yeast and mold counts, low lactic acid bacteria counts, low total plate counts, and no visible mold spoilage after five weeks at 15°C, predicting a minimum shelf life of ten weeks at 5°C. Considering the multiple successes, the acid system of citric and acetic acids in conjunction with 20 ppm natamycin was trialed at 5°C at pH 4.6 and pH 4.2 in Phase II to assess real-time refrigerated shelf life.

3.2 Phase II – Real-time Refrigerated Storage

3.2.1. Microbial Enumeration – Yeasts and Mold

The results of yeasts and mold (YM) counts from pH 4.6 citric-acetic hummus samples stored at 5°C are presented in **Figure 2.6A**. Similar to the results from the accelerated study, the control hummus samples had visible signs of mold spoilage and high YM counts by Week 2, and all samples were spoiled with *Penicillium* by Week 4 (counts $>10^6$ CFU/g hummus). Also mirroring the accelerated study, the preservative potassium sorbate maintained low YM counts through Week 4, but all samples had spoiled with *Penicillium* by Week 6. Comparing the real-time storage results to the accelerated storage results, shelf life was twice as long at 5°C as it was at 15°C, confirming a Q_{10} value of 2. Another chemical preservative, sodium benzoate, had similar results as potassium sorbate, with increased YM counts by Week 4 and all sodium benzoate samples spoiled with *Penicillium* by Week 10.

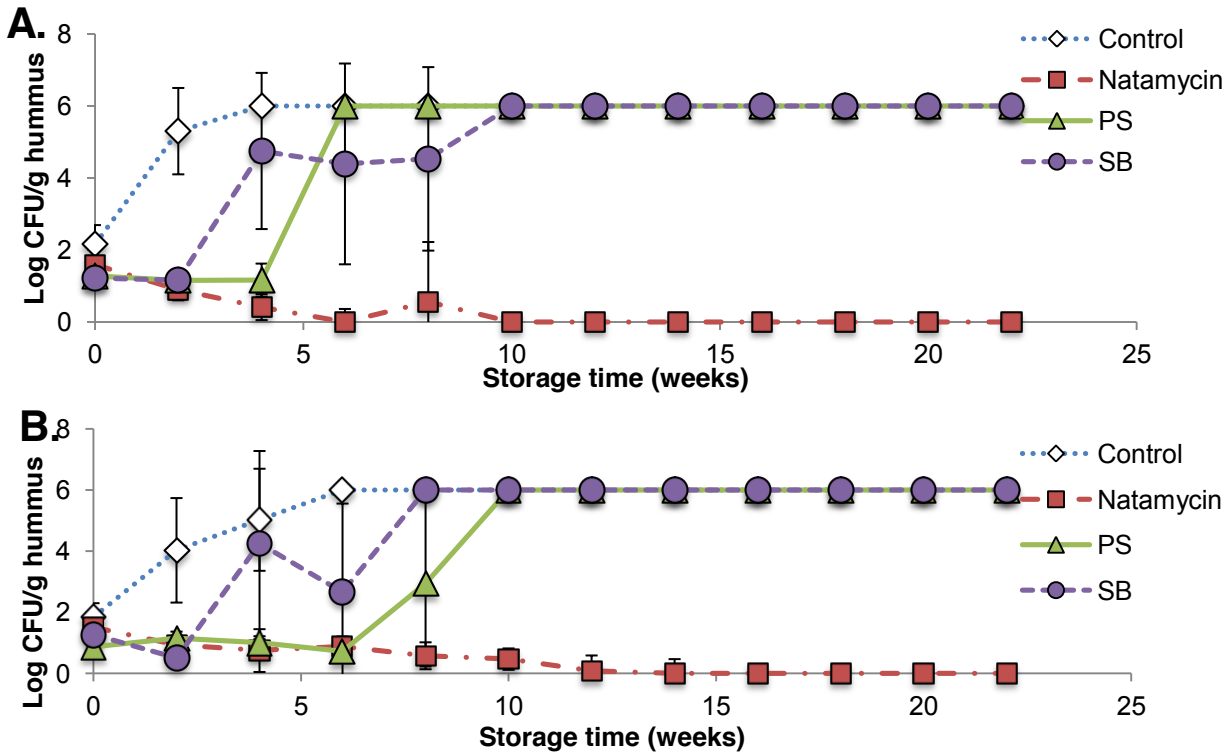

Figure 2.6 Changes in yeast and mold counts in citric-acetic hummus treated with different antimicrobials over storage period at 5°C; A) pH 4.6 B) pH 4.2 *[in legend, PS = potassium sorbate, SB = sodium benzoate]*

In contrast, YM counts decreased in natamycin-treated samples (**Figure 2.6A**). Natamycin YM counts were significantly lower than control YM counts ($p < 0.01$) from Week 2 onward and were significantly lower than all other samples from Week 6 onward ($p < 0.01$). No visible mold spoilage has been observed on any natamycin-treated hummus samples at time of publication (Week 22), excluding three cups that had mold growth on one side. Due to insufficient stirring and the inoculum being concentrated in one are, natamycin was likely unable to control mold growth in those three samples.

By Week 10, there were no yeast or mold colonies observed on the lowest dilution plate of 10^{-1} for natamycin-treated samples. Thus, it was reported that there was <1 CFU/g hummus (limit of detection) for the continuation of the study, which implied that no mold survived from the initial inoculum level of 10^{2} CFU/g. These results confirm observations from the accelerated

study that natamycin has a fungicidal effect against *Penicillium* at pH 4.6 with citric and acetic acids. The fungicidal effect of natamycin against various yeasts and molds has been previously reported in use with food biofilms (Ture et al. 2011, Olle Resa et al. 2013), in dry sausages as a surface spray (Pipek et al. 2010), and in Niagara grape juice (Siricururatana et al. 2013). This study, however, is the first report of its success when incorporated as an ingredient during food manufacture and demonstrates a sustained fungicidal effect throughout shelf life, even in complex food matrices with high A_w and fat content.

Figure 2.6B present the YM counts from pH 4.2 citric-acetic hummus samples stored at 5°C. The control and sodium benzoate-treated samples had very similar levels of YM counts at pH 4.2 as at pH 4.6. Potassium sorbate, however, had improved performance at the lower pH, as it was not until Week 10 that all samples spoiled. The improved antimicrobial activity of potassium sorbate at pH 4.2 is expected, as its pK_a of 4.75 would indicate that a greater proportion of the molecules would be present in its undissociated form at a lower pH (Yamani and Mehyar 2011). Thus, it would be easier for the undissociated molecule to enter microbial cells and then dissociate to induce its antimicrobial effect internally, thereby inhibiting spoilage.

Similar to the results of hummus at pH 4.6, the natamycin-treated pH 4.2 hummus samples had decreasing YM counts throughout 5°C storage (**Figure 2.6B**). Natamycin YM counts were significantly lower than control YM counts ($p < 0.05$) from Week 2 onward, and natamycin YM counts were significantly lower than all other samples from Week 10 onward ($p < 0.01$). By Week 12, YM counts of natamycin samples were significantly lower ($p < 0.05$) than YM counts at Week 0, which provides further evidence of natamycin's fungicidal effect. No visible mold spoilage has been observed on any of the natamycin-treated hummus samples at time of publication (Week 22). Although no *Penicillium* colonies were observed on the growth

media after Week 4, there was a population of *Aspergillus* that maintained YM counts above the detection limit for several additional weeks. It is likely that the *Aspergillus* came from a contaminated ingredient, as it was present in all three natamycin replicates at pH 4.2. Nevertheless, by Week 20, all natamycin-treated hummus samples at pH 4.2 had YM counts below the detection limit (<1 CFU/g), demonstrating fungicidal effect against *Aspergillus*.

It is concluded that the combination of natamycin with citric and acetic acids is the most effective acid-antimicrobial formulation for controlling yeast and mold growth in hummus at both pH 4.6 and pH 4.2.

3.2.2. Microbial Enumeration – Lactic Acid Bacteria

Very low initial LAB counts were enumerated (<1 log CFU/g, data not shown) in all samples for both pH 4.6 and pH 4.2 hummus batches, regardless of antimicrobial treatment. Given such low counts, LAB enumeration was repeated only once every four weeks for the remainder of storage to monitor if any population changes occurred. At time of publication, LAB counts were below the detection limit (<1 CFU/g) for all samples. These results corroborate the results from the accelerated study that the combination of citric and acetic acids effectively inhibits growth of LAB in hummus during refrigerated storage.

3.2.3. Microbial Enumeration – Total Plate Count

The results of total plate count (TPC) of pH 4.6 and pH 4.2 citric-acetic hummus samples stored at 5°C are presented in **Figure 2.7A** and **2.7B**, respectively. Patterns of growth were very similar to YM growth for each corresponding antimicrobial treatment, as previously discussed. Prior to being spoiled with mold, the TPC of control, potassium sorbate, and sodium benzoate samples were approximately the same as YM counts, indicating low bacterial growth at both pH.

With the natamycin samples, mean TPC values steadily decreased during storage for hummus at both pH, which also reflected the decrease in YM counts. By Week 12, TPC of pH 4.2 natamycin samples were significantly lower ($p < 0.01$) than TPC at Week 0. As bacteria were initially present in the pH 4.2 samples, these results indicate that a bactericidal effect was observed. In contrast, TPC of pH 4.6 natamycin samples were static and had no significant change ($p > 0.05$) over the storage period, despite decreases in YM counts. As YM counts were not significantly different ($p > 0.05$) between the two pH groups with natamycin at Week 12 or beyond, the difference in TPC is likely attributed to higher bacterial counts in pH 4.6 hummus. Thus, the bactericidal effect observed in the pH 4.2 natamycin hummus was not present at pH 4.6. This bactericidal effect may have been caused by the acetic acid present, as it is a weak acid and has been reported by other researchers to inhibit growth of various bacteria and pathogens (Asensi et al. 1997, Khurana et al. 2006, Sullivan et al. 2013). Its low pK_a of 4.7 means that a larger percentage of the acid will be its un-dissociated form at pH 4.2 compared to pH 4.6, and thus a greater bactericidal effect can be conferred.

As the antimicrobial mechanism of natamycin is due to its specific interaction with ergosterol (te Welscher et al. 2010), a compound specific to fungi, natamycin is not thought to be active against bacteria. However, a decrease in TPC was not observed in citric-acetic hummus alone, so the antibacterial effect may be from a synergistic activity between the two acids and natamycin. Such antibacterial activity with natamycin in a low-pH environment was also observed in Galotyri cheese (Kallinteri et al. 2013), where natamycin was more effective in lowering LAB counts than nisin during refrigerated storage over 28 days. Studies investigating the antibacterial activity of natamycin are limited, although one report found natamycin to have a small but significant inhibitory effect on bacteria isolated from soil (Mohamed et al. 2005).

Further research is necessary to quantify the inhibitory effects of natamycin on bacteria, particularly with bacteria of relevance to food safety.

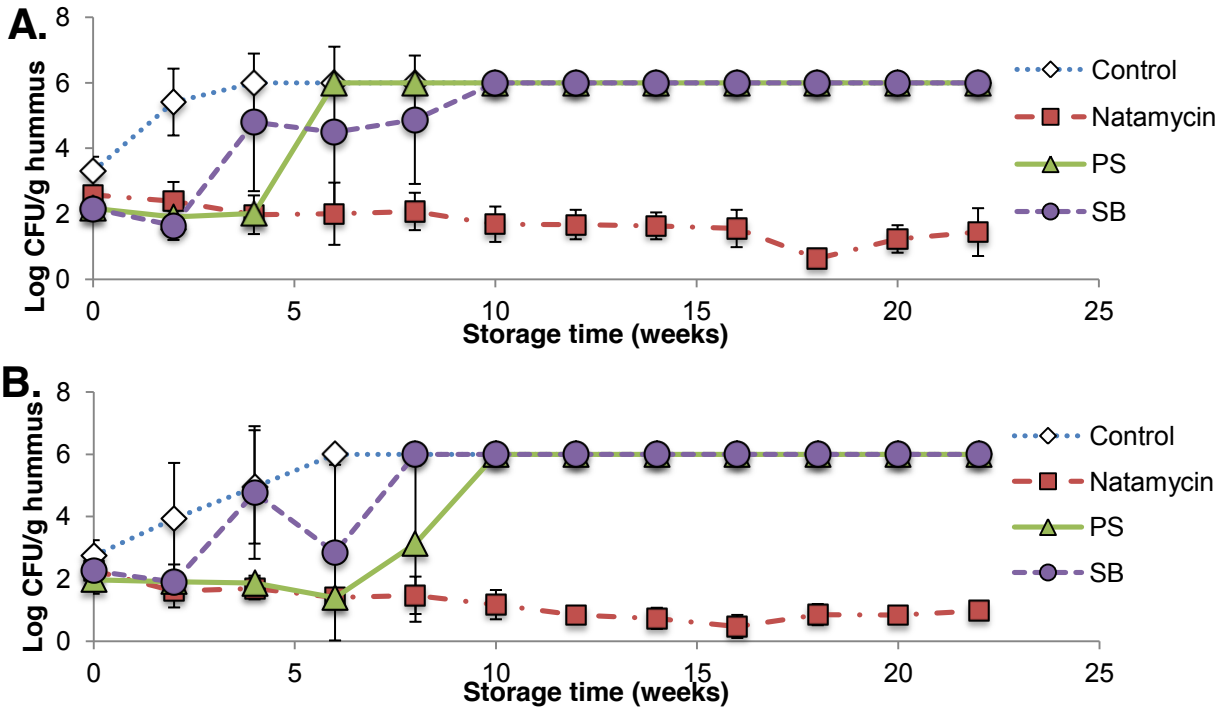

Figure 2.7 Changes in total plate counts in citric-acetic hummus treated with different antimicrobials over storage period at 5°C; A) pH 4.6 B) pH 4.2 *[in legend, PS = potassium sorbate, SB = sodium benzoate]*

Overall, the total plate counts were low in all unspoiled samples, confirming that the combination of citric and acetic acids are successful in maintaining low bacterial counts. Natamycin was the most successful preservative in maintaining low TPC of the treatments tested.

3.2.4. Changes in pH

The pH of hummus samples was analyzed biweekly, and only unspoiled samples were measured. With all antimicrobial treatments and at both pH, the hummus pH was approximately constant at the target pH value for unspoiled samples throughout all of shelf life. These results are consistent with the low microbial counts prior to mold spoilage. Therefore, it is concluded that citric-acetic acid hummus is stable with regards to pH.

3.2.5. Prediction of Shelf Life

Table 2.7 displays the determined shelf life of the hummus samples in refrigerated storage (5°C). Control samples had the shortest shelf life at both pH, and although shelf life increased at the lower pH, it was not a significant increase from pH 4.6 ($p > 0.05$). Potassium sorbate samples had significantly longer shelf life at pH 4.2 (62 days) compared to pH 4.6 (38 days, $p < 0.01$). Sodium benzoate samples had no significant difference ($p > 0.05$) in shelf life between the two pH. As the pK_a of benzoic acid is 4.2, its antimicrobial activity improves at pH<4.0, so its limited inhibitory success at the pH values tested was expected. From these results, lowering the pH of citric-acetic hummus to pH 4.2 improves shelf life compared to pH 4.6 when using potassium sorbate, but not with sodium benzoate or no antimicrobial treatment. At pH 4.6, there was no statistically significant difference in shelf life between control samples and samples treated with chemical preservatives ($p > 0.05$), though the 20-day increase in shelf life with the chemical preservatives might be significant from a practical perspective. The determined shelf life values for control and chemically-preserved hummus are comparable to those found by Yamani and Mehyar (2011) at pH 4.5. At pH 4.2, potassium sorbate samples had a significantly longer shelf life than the control ($p < 0.05$) while sodium benzoate samples' shelf life did not differ significantly from either the control or potassium sorbate samples ($p > 0.05$).

Table 2.7 Determined shelf life of hummus samples in refrigerated storage (5°C)

	Shelf Life in Days (Mean ± SD)			
Hummus pH	**Natamycin**	**Control**	**Potassium Sorbate**	**Sodium Benzoate**
pH 4.6	$>154^{A}$	$19 \pm 8^{a,B}$	$38 \pm 7^{a,B}$	$43 \pm 16^{a,B}$
pH 4.2	$>154^{A}$	$28 \pm 14^{a,C}$	$62 \pm 8^{b,B}$	$45 \pm 2^{a,BC}$

[a,b] Values in the same column with different letters are significantly different ($p < 0.05$)
[A-C] Values in the same row with different letters are significantly different ($p < 0.05$)

As no natamycin samples at either pH spoiled during the 22 weeks of refrigerated storage, it is determined that natamycin-treated citric-acetic hummus has a shelf life of over 154 days, which is approximately equivalent to five months. The shelf life is significantly longer than all other treatments, both at pH 4.2 and pH 4.6 ($p < 0.01$). This shelf life is also longer than the best shelf life achieved by Yamani and Mehyar (2011) with a combination of potassium sorbate and sodium metabisulfite in hummus (only 90 days). However, the shelf life in our study was determined solely by microbial parameters, and samples were not assessed for organoleptic qualities. Depending on the formulation and additional ingredients, the mode of failure for shelf life with natamycin-treated citric-acetic hummus may most likely be undesirable changes in texture or flavor instead of microbial spoilage. Therefore, while using natamycin with citric and acetic acids can obtain a refrigerated shelf life of five months, hummus manufacturers should conduct organoleptic assessments of samples to determine the shelf life for optimal quality.

It should be noted that the inoculum level used in these storage studies was greater than would be expected in manufacturing facilities that follow good manufacturing practices (GMPs). The shelf life period determined for the control, potassium sorbate, and sodium benzoate may be considered "worst case" scenarios. Nevertheless, the study was designed to demonstrate whether these treatments could successfully control microbial growth even in severe cases, which revealed significant success for natamycin and less promise for the other treatments. Thus, the challenge with *Penicillium* clearly identified natamycin as a key ingredient for extending shelf life in hummus.

3.2.6. Sensory Discrimination Test – Triangle Test

With the success of citric and acetic acids in extending the shelf life of hummus with natamycin, this two-acid system was compared against citric acid alone in a triangle test.

Untrained panelists were instructed to taste two sets of hummus sample triads and identify the odd sample. Of the 42 participants in the triangle test, there were 17 correct responses for the pH 4.6 hummus set and 17 correct responses for the pH 4.2 hummus set (**Table 2.8**). The critical value for correct responses at $p = 0.05$ with 42 panelists is 20 (Lawless and Heymann 2010). Thus, there was no significant difference perceived between citric acid hummus and citric-acetic acid hummus at either pH. This indicates that the majority of consumers would not notice any flavor change if a hummus manufacturer were to replace 20% of citric acid with acetic acid in their formulation to extend shelf life. Even at higher levels of acidity in pH 4.2, the addition of acetic acid did not result in a perceivable difference, which is very promising for applications that may require lower pH formulations such as thermal processing for shelf-stable products.

Table 2.8 Correct responses from panelists of hummus triangle test

Hummus Set	*N* = Panelists	Correct Responses	% Correct	Correct Responses Required for Significance ($p < 0.05$)
Set 1: pH 4.6	42	17	40%	20
Set 2: pH 4.2	42	17	40%	20

It is important to recognize that although the addition of acetic acid did not result in a significant flavor difference, there are likely consumers who would perceive a difference. Out of the 42 panelists, nine (21%) correctly identified the odd sample in both sets, and these panelists are most likely discriminators who recognize the true difference in the hummus and would notice the change in a commercial product (Lawless and Heymann 2010). It would be up to the hummus manufacturer to decide if approximately 20% of consumers perceiving this change would be detrimental to consumer acceptability and how to mitigate such perceptions. Potential solutions for minimizing flavors changes with added acetic acid could be increased tahini or olive oil levels, or adding new ingredients for a different flavor.

3.2.7. Collected Conclusions from Phase I & II and Next Steps for Phase III

During the accelerated shelf life study at 15°C, the combination of citric and acetic acids with natamycin demonstrated a better inhibition of microbial growth in hummus compared to citric acid, GDL, and GDL plus acetic acid with any other antimicrobial. With the refrigerated storage study, the citric-acetic natamycin hummus samples at both pH 4.2 and pH 4.6 lasted 154, or approximately five months, at 5°C without visible mold spoilage. This shelf life was significantly longer ($p < 0.01$) than the control, potassium sorbate, or sodium benzoate samples at either pH. Sensory evaluation with untrained panelists confirmed that citric-acetic acid hummus is not perceivably different from citric acid hummus. It is concluded that the combination of citric and acetic acids with natamycin successfully extends the shelf life of refrigerated hummus to 154 days, or five months, and this acid-antimicrobial combination is suggested as a viable option for manufacturers that seek to use natural ingredients.

Furthermore, the antimicrobial effects of natamycin with citric and acetic acids may help extend shelf life for shelf-stable hummus products as well. The application of this acid-antimicrobial formulation was applied to thermal processing of shelf-stable hummus in the third and final phase of this project.

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CHAPTER 3
EFFECT OF ANTIMICROBIALS ON PRODUCTION OF ACIDIFIED SHELF-STABLE HUMMUS BY HOT-PACKING

1. INTRODUCTION

In the United States, hummus dip products are typically sold in the refrigerated section in sealed polyethylene terephthalate (PET) tubs and must be kept refrigerated throughout distribution and storage. However, there is interest in developing shelf-stable hummus products to expand distribution chains into the shelf-stable category. The popularity of hummus with U.S. consumers indicates that there is market opportunity for hummus products that break the boundary of refrigeration (Perkowski 2014), creating a more convenient product. In the Middle East, shelf-stable canned hummus products are common, but heating regimes are very harsh, requiring 32.9 and 57.2 min at 121°C for small and medium-sized cans, respectively, with a filling temperature of 85°C, using F_0 = 2.78 min (Amr and Yaseen 1994). Aseptic processing employs very high temperatures (93°C to 140°C, depending on pH) for a few seconds (2 to 15 s), and this method has recently been successfully applied to hummus (Jed 2012). Yet both aseptic and retort equipment are expensive investments and have large energy costs, which are major concerns for small-scale producers. If lower temperatures could be used in combination with other hurdles to achieve shelf-stability, thermal processing of hummus would be more accessible to smaller producers and potentially more appealing to consumers.

A thermal treatment option to produce shelf-stable foods is known as “hot-fill hold”, a process commonly used for acidified foods with pH < 4.6. This method involves heating the product, filling the hot product into the final containers, sealing the containers, and holding for a prescribed period of time. The heat from the product must be enough to also heat the container during the hold time so that the package is sterilized as well (GMA-SEF 2007a). Typically, the

amount of headspace in the container is also specified to ensure adequate vacuum, and the entire process must be reviewed and approved by a process authority. Being that the hot-fill hold method does not require elaborate equipment, it is feasible for small-scale producers to complete.

According to the U.S. Code of Federal Regulations, Title 21 Part 114.8E, commercially sterile foods must have undergone a thermal process that ensures the food is a) free of microorganisms capable of reproducing under normal storage conditions and b) free of viable pathogens (including spores). Current recommendations for hot-filling a food product at $pH \leq 4.2$ are to heat to 93.3°C and hold for five minutes to ensure that the product is commercially sterile (Pflug 1998). Heating hummus to such a high temperature may negatively impact flavor or texture qualities. Utilizing antimicrobials in a hummus formulation may act as an additional hurdle against microbial growth. From the results of the refrigerated hummus study, it was identified that the combination of citric and acetic acids in hummus treated with natamycin demonstrated very strong antimicrobial effects. These hurdles could be potentially combined to produce a shelf-stable hummus product that is processed at lower temperatures to minimize negative effects on sensorial or nutrient aspects (Leistner 2000).

The objective for this project segment is to determine the processing temperature(s) (76.7°C, 82.2°C, or 87.8°C) that produce a shelf-stable hummus product utilizing the hot-fill hold method. The hummus formulation of acidification to pH 4.2 with citric and acetic acids will be studied with no antimicrobial (control), 20 ppm natamycin (natural preservative) and 1000 ppm potassium sorbate plus 1000 ppm sodium benzoate (chemical preservatives). All systems were challenged with mold inoculation. From the results of physical attribute analyses and microbial enumeration, the best temperature-formulation combination will be identified for the production of shelf-stable hummus.

2. MATERIALS AND METHODS

2.1 Materials for Hummus Preparation

The same materials and ingredients listed in Chapter 2, Sections 2.1.1 and 2.2.1 were also used for hummus preparation in the shelf-stable trials.

2.2 Hummus Preparation

Batches of 15.0-kg hummus were prepared in the Fruit & Vegetable Pilot Plant at the New York State Agricultural Experiment Station (Geneva NY). Formulations are detailed in **Table 3.1**. Chickpeas were drained from the cooking liquid and rinsed in cold water prior to being ground into a smooth paste with a meat grinder (Model 2822, U.S. Berkel/U.S. Slicing Machine Company Inc., Laporte, IN). The chickpea paste was transferred to the mixing bowl of a 60-qt. H660T mixer (Hobart Manufacturing Company, Troy, OH), at which point tahini, olive oil, and sodium chloride were added and thoroughly mixed. The hummus was then acidified by addition of the appropriate level of citric and acetic acid, detailed in **Table 3.1**, to achieve the target pH of 4.20. Higher amounts of acid were added for the potassium sorbate and sodium benzoate batch because of the basic nature of those ingredients. Subsequently, one of three antimicrobial treatments was added: no antimicrobial agent added (control), 40 ppm Natamax® (equivalent to 20 ppm natamycin), or 1000 ppm potassium sorbate with 1000 ppm sodium benzoate. In the case of Natamax®, the Natamax® powder was mixed with the portion of sodium chloride prior to addition to adequately disperse the powder. Water was added to reach 100% of formulation.

Table 3.1. Formulations of 15.0-kg citric-acetic hummus batches in pilot plant; A) Control, B) Natamycin, and C) Potassium Sorbate and Sodium Benzoate

A) Control

Ingredient	Percentage (% w/w)
Chickpeas	74.0
Tahini	4.0
Olive oil	10.0
Table Salt (NaCl)	0.5
Citric Acid (50% solution)	0.56
Acetic Acid (5% solution)	0.112
Water	10.8
Total	**100**

B) Natamycin

Ingredient	Percentage (% w/w)
Chickpeas	74.0
Tahini	4.0
Olive oil	10.0
Table Salt (NaCl)	0.5
Citric Acid (50% solution)	0.56
Acetic Acid (5% solution)	0.112
Natamax (50% natamycin)	0.004
Water	10.8
Total	**100**

C) Potassium Sorbate + Sodium Benzoate

Ingredient	Percentage (% w/w)
Chickpeas	74.0
Tahini	4.0
Olive oil	10.0
Table Salt (NaCl)	0.5
Citric Acid (50% solution)	0.66
Acetic Acid (5% solution)	0.112
Potassium sorbate (15% solution)	0.1
Sodium benzoate (15% solution)	0.1
Water	10.5
Total	**100**

2.3 Mold Inoculation

The same mold inoculum of *Penicillium* was used as previously described for the refrigerated hummus study in Chapter 2. In addition, a mold inoculum of *Aspergillus niger* was used in conjunction with the *Penicillium* inoculum. *Aspergillus niger* is another common spoilage organism in food products and had been observed in some hummus samples from the

refrigerated study. Neither mold is heat-resistant in the stage of asexual spores and do not survive typical pasteurization processes (Pitt 2006). However, it was chosen to combine *A. niger* and *Penicillium* as inocula for the thermally-treated hummus trials to further challenge the system. The stock of *A. niger* was supplied by the laboratory of Randy W. Worobo at Cornell University (Geneva, NY) and the inoculum suspension was prepared in the same manner as described for *Penicillium* in Section 2.1.3. The *A. niger* stock suspension was determined to have approximately 1.96 x 10^8 CFU/mL

To inoculate prior to thermal treatment, 11.25 mL of the 10^{-2} dilution of *Penicillium* and 7.80 mL of the 10^{-3} dilution of *A. niger* inoculum suspensions were added to the 15-kg hummus batch in the mixing bowl, achieving an inoculation level of approximately 100 CFU/g hummus for each mold. The hummus was then mixed for two minutes at Speed 3 on the Hobart mixer to ensure thorough incorporation of the mold inocula. High-speed mixing was used conservatively to minimize air incorporation into the product. At this point for each batch, three 6-oz. jars were filled with hummus for later analysis as a "pre-thermal" sample.

2.4 Thermal Treatment in Scraped-Surface Heat Exchanger

A scraped-surface heat exchanger (SSHE) was chosen for thermal treatment of the hummus because of its superior handling of viscous products that would likely foul on the heat transfer surface (Rao & Hartel 2006). A Votator® X1W SSHE was employed for processing the hummus, connected to a progressing cavity pump (Type SSQ, Frame F3, Form MR; Monyo Inc., Springfield OH) with an open-throat auger funnel feed. The scraper blade speed of the SSHE was set to 1800 rpm, the highest speed available on the equipment. The pump belt speed was set to 5. For more precise control of the feed flow through the pump, a variable frequency drive (AC Drive V7-4X, Yaskawa America Inc, Waukegan IL) was connected to the pump control

panel and set to 16.5 Hz. Steam was used as the heating medium, supplied by a 60 lb. steam pipe. Water at room temperature was first pumped through the SSHE, and the steam pressure was controlled to reach the target temperature range. The feed was then switched to the prepared inoculated hummus, and the steam pressure was adjusted to stabilize at the target processing temperature. Three fill temperatures were tested – 76.7°C, 82.2°C, and 87.8°C – with each of the three antimicrobial treatments of hummus, thereby resulting in nine trials. The temperature of the processed hummus was measured at the outlet tube of the SSHE with a handheld thermocouple (Atkins AquaTuff® 351, Cooper-Atkins Corp. Middlefield CT) and confirmed to be the target processing temperature ±0.5°C prior to filling. Jars and lids that were to be used for packaging were pre-heated in boiling water.

The thermally-treated hummus was hot-filled from the outlet tube into individual 6-oz. clear glass jars, capped with a plastisol-lined continuous thread lid, and inverted immediately. The jars were inverted for five minutes and then turned right-side up to air-cool to room temperature. Jars were filled to a level leaving approximately 10% headspace. Twelve jars were collected for each treatment-temperature combination. As each jar was hot-filled independently, each jar is considered an independent replicate.

2.5 Vacuum, Viscosity, and pH Measurements

From the twelve jars per trial of thermally processed hummus, three jars were selected at random for vacuum testing, viscosity analysis, and pH measurement. All measurements were performed after the samples had equilibrated to room temperature (20°C) for 12 hours.

Vacuum quality of each thermally processed hummus jar was evaluated by measuring the interior pressure with a vacuum gauge. The center of the lid was punctured with a piercing

device (Zahm Nahl Co. Inc., Buffalo NY) connected to a pressure gauge with a rubber seal. Pressure measurements were recorded in inches mercury.

To obtain viscosity measurements, steady-shear experiments were performed using a Brookfield DV-III Ultra Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro MA) with a V-74 vane spindle, externally controlled with Rheocalc software (Brookfield Engineering Laboratories, Inc., Middleboro MA). The hummus viscosity was analyzed directly in the 6-oz. jar. Steady-shear experiments were conducted based on the protocol of Verlent et al. (2006). The spindle was lowered into the center of the sample, and steady-shear tests were carried out using shear rates from 0.11/sec to 0.63/sec. The duration of each measuring point was 60 seconds, and a total of 11 points were collected for each sample. Both pre and post thermally processed hummus samples were tested for each temperature-treatment combination. Shear stress (τ [D/cm^2]) and shear rate (D [1/sec]) data were applied to the Power Law function to determine the consistency index for viscosity (k [cP]) and the flow index (n):

$$\tau = kD^n$$

Both pre and post thermally processed hummus samples were measured for pH. All pH measurements were made as previously described in Chapter 2, Section 2.1.6.

2.6 Microbial Sampling

Three jars per heating trial were analyzed to measure the initial microbial load after the samples had equilibrated to room temperature (20°C) for 12 hours. Yeasts and molds were enumerated on acidified potato dextrose agar (PDA), lactic acid bacteria were enumerated on deMan Rogosa Sharpes (MRS) agar, and total plate counts were enumerated on standard plate count agar (PCA). Sampling, plating, and enumeration methods were followed as previously described in Chapter 2, Section 2.1.5.

2.7 Incubation of Thermally Processed Hummus and Product Inspection

Six jars per thermal trial were incubated at 35°C for 10 days, as outlined in the USDA-FSIS regulations (9 CRF 318.309 and 9 CRF 381.309) for finished product inspection. The incubated jars were visually inspected every day for abnormalities (e.g. swelling jars, bulging lids, discoloration of hummus). If no visual abnormalities were observed after 10 days, then the sample was removed from incubation and tested for measurement of the microbial load. Yeasts and molds were enumerated on acidified PDA, lactic acid bacteria were enumerated on MRS agar, and total plate counts were enumerated on PCA. Sampling, plating, and enumeration methods were followed as previously described in Chapter 2, Section 2.1.5.

2.8 Long-Term Storage

Three jars from each heating trial were placed in long-term storage at 18°C. Jars were visually inspected once every two months for abnormalities. Results of the incubated samples were to be corroborated by the long-term storage study.

2.9 Determination of Lethality Values

To determine the lethality values of the process regimes at 76.7°C, 82.2°C, and 87.8°C in comparison to processing at 93.3°C, heat penetration data were collected. Due to the intricacy of the heat penetration collection system, the thermal processes were replicated on benchtop instead of testing during the pilot plant trials. Jar lids were fitted with two thermocouples: one straight probe in the lid's center and one flexible thermocouple on the lid's side, both measuring the temperature at mid-depth of the jar. The thermocouples were connected to a CalPlex Datalogger (TechniCAL Inc., Metairie LA) that was controlled by CalSoft5 (TechniCAL Inc., Metairie LA). Thermocouples were calibrated to 100°C against a handheld thermocouple. The 6-oz. glass jars were prewarmed in boiling water and removed just prior to filling.

Hummus was prepared in the pilot plant as previously described, following the natamycin formulation in **Table 3.1**. The same batch of hummus was used for every trial. Hummus was heated in a microwave (Radarange RFS10 2250 Watt, Amana®, Benton Harbor MI) on high power for 1.5 minutes and then in 20-second intervals until the target fill temperature of 76.7°C, 82.2°C, or 87.8°C was reached. The temperature of the hummus was verified with the handheld thermocouple. The hummus was then poured into the pre-warmed jar, the lid with thermocouples was capped, and the jar was inverted. Jars were filled to a level leaving approximately 10% headspace as conducted in the pilot plant trials. Temperature recording was initiated just prior to filling, with the start time of inversion noted, and temperature values were collected every 10 seconds for ten minutes after the start of inversion. Five replicates were completed for each target fill temperature.

Lethality values were calculated at each data collection point using the following equation from the model of Bigelow (Pflug 1982):

$$F(T_{ref}) = \Delta t \times 10^{(\frac{T-T_{ref}}{z})}$$

Where T_{ref} = 93.3°C, T = temperature in °C measured at that time point, z = 8.9°C, and Δt = time in minutes since the previous temperature recording, which is assumed to be the time that the product was held at T. The lethality values were totaled over 5 min. and 10 min. to calculate the accumulated lethality of the thermal process in reference to processing at 93.3°C.

Section 2.10 – Statistical Analysis

Vacuum, viscosity, and lethality value measurements were analyzed by one-way analysis of variance (ANOVA). Total plate counts were analyzed by the Student t-test for significant changes after the incubation period. All statistical analysis was completed using JMP 11.0 Statistical Software (Cary, NC). Statistical significance was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Vacuum, Viscosity and pH Measurement Results

The mean values of vacuum at each processing temperature are presented in **Table 3.2**. As three jars were sampled from each batch at each processing temperature, the values are an average of nine measurements. The vacuum achieved at the 87.8°C fill temperature is significantly higher ($p < 0.05$) than the vacuums achieved at either 76.7°C or 82.2°C. Given that all other factors (headspace, air in product, capping technique) were held constant, it is expected for the jars from the highest fill-temperature to have the highest vacuum (GMA-SEF 2007b). All jars (including those processed at the lower temperatures) were above the minimum vacuum requirement of 6 inches Hg and within the range of other reported vacuum measurements reported (Tucker & Featherstone 2011). Thus, under the processing conditions tested, a hermetic seal was obtained.

Table 3.2 Measurements of vacuum and viscosity of hot-packed hummus

	Mean ± SD	
Fill Temperature	**Vacuum (inches Hg)**	**Viscosity (Pa·s)**
76.7°C	7.5 ± 2^{a}	160 ± 30^{a}
82.2°C	8.4 ± 1^{a}	130 ± 40^{a}
87.8°C	9.9 ± 2^{b}	150 ± 50^{a}

Values in the same column with different letters are significantly different ($p < 0.05$).

Viscosity was calculated from steady shear stress and shear rate experiments, applying the Power Law function as discussed in Section 2.5. When grouped by fill temperature, there was no significant difference ($p > 0.05$) among the final hummus viscosities processed at different temperatures. However, when all thermally processed hummus viscosities were grouped together and compared against the pre-thermally treated hummus viscosity, there was a statistically significant increase ($p < 0.05$) in the heated hummus viscosity (**Table 3.3**). The

thermally processed hummus also had a significantly lower (more negative, $p < 0.05$) flow index, indicating that heating the hummus makes it more pseudoplastic (shear-thinning).

Table 3.3. Measurements of viscosity, flow index, and confidence of fit from pre and post thermally processed hummus as determined by the Power Law

	Mean ± SD	
	Pre-thermal	Post-thermal*
Viscosity (Pa·s)	120 ± 20[a]	150± 40[b]
Flow Index	-0.21 ± 0.06[a]	-0.32 ± 0.10[b]
Confidence of Fit (%)	70 ± 5[a]	69 ± 8[a]

*Post-thermal refers to the mean of all values for hummus processed at 76.7°C, 82.2°C, and 87.8°C. Values in the same row with different letters are significantly different ($p < 0.05$).

The change in hummus viscosity after thermal processing was most likely from structural changes of proteins in the food matrix. Although the chickpeas were already retorted and proteins were previously denatured, the acidification of the hummus altered the chemical environment and likely exposed different protein side chains. When followed by thermal treatment, new protein conformations could have occurred, resulting in increased viscosity. Cai and Baik (2001) investigated chickpea protein interactions and found that heating at pH < 5.2 promoted hydrophobic interactions, which could account for the viscosity changes observed in the hummus. Amr and Yaseen (1994) also observed increased viscosity during thermal treatment of hummus and noted that the sesame proteins in tahini may have been denatured, causing viscosity increases. In our formulations, tahini only accounted for 4% of ingredient weight, but these proteins could have had a plausible effect, especially if interacting with chickpea proteins.

The observed changes in hummus viscosity during thermal processing were not unexpected, but it is unknown how the increase in viscosity will impact consumer preferences of texture. Sensory evaluation conducted by Amr and Yaseen (1994) found parity of consumer acceptability of the heat-treated hummus to the non-treated control, even though the heat-treated product was significantly more viscous. However, the study was conducted with Jordanian

consumers who are accustomed to canned hummus products in the Middle East. For manufacturers seeking to produce shelf-stable hummus in the U.S., future research involving sensory evaluations of thermally treated hummus would be beneficial to determine the acceptability of the thicker texture.

All pH measurements of hummus samples, including prior to thermal treatment, after thermal treatment and after incubation at 35°C, were equivalent to pH 4.2 ± 0.05. Such variations are expected from day-to-day differences with pH electrodes and indicate that the hummus pH was maintained both during processing and product incubation.

3.2 Product Incubation at 35°C and Inspection

None of the jars from any of the nine temperature-antimicrobial treatments showed signs of swelling or bulging after 10 days of incubation at 35°C. Under USDA-FSIS regulations (9 CRF 318.309 and 9 CRF 381.309), all samples would therefore pass finished product inspection for safety. Thus, all hummus samples from incubation were analyzed for microbial enumeration to better assess the quality of the products.

3.3 Microbial Enumeration – Yeasts and Mold

Initial yeast and mold (YM) counts were analyzed from thermally treated hummus samples within 12 hours of the jars reaching room temperature. None of the samples had yeast or mold growth on acidified PDA plates of lowest dilution (10^{-1}), regardless of fill temperature or antimicrobial treatment. This uniform result across all temperatures indicates that even the lowest thermal treatment (76.7°C) had successfully eliminated the mold inocula and any other fungal microbes that may have been present in the hummus at time of processing.

However, after 10 days of incubation at 35°C, YM counts were more varied across treatments. Five out of six incubated hummus samples from the 76.7°C control treatment had mold colonies on PDA plates, and in one jar, mold mycelia was visible on the hummus surface upon opening the jar (spoilage results in **Table 3.4**). All incubated hummus samples from the 82.2°C control treatment had mold growth on PDA plates, and in two jars, mold mycelia was visible on the hummus surface. None of the incubated samples from the 87.8°C control treatment had mold growth.

The hummus samples formulated with preservatives had lower incidences of mold growth. Two out of six incubated hummus samples from the 76.7°C natamycin treatment had mold colonies on PDA plates, but none had visible mold growth on the hummus surface. None of the incubated samples from the 82.2°C or 87.8°C natamycin treatment had mold growth. With the potassium sorbate + sodium benzoate incubated samples, none had mold growth at any of the fill temperatures.

Table 3.4. Mold spoilage of acidified, hot-packed hummus after incubation at 35°C for 10 days

Fill Temperature	Jars with Mold Growth*			
	Control (out of 6)	Natamycin (out of 6)	Potassium sorbate + sodium benzoate (out of 6)	Total Spoiled (out of 18)
76.7°C	5	2	0	7
82.2°C	6	0	0	6
87.8°C	0	0	0	0
Total Spoiled (out of 18)	11	2	0	13

*If mold colonies were enumerated on acidified PDA, then the sample was classified as having mold growth. All unspoiled samples had no detected mold growth on acidified PDA.

It was noted that all mold colonies from the spoiled incubated hummus samples were identical in morphology and were neither *Penicillium* nor *Aspergillus niger*. From microscopic observation of the colonies, the mold was hypothesized to be a species of *Moniliella*, a yeast-like mold that has been reported to survive weak-acid preservatives (Pitt and Hocking 2009). This

observation implicates the cause to be either contamination from one of the ingredients or from post-thermal contamination during packaging. Although all lids and jars were treated in boiling water, it is possible that contamination from the processing environment may have occurred after they were removed from the steam kettle and before being filled with the hot hummus product. This stresses the importance of clean processing environments and adherence to good manufacturing practices (GMPs). If the mold came from a contaminated ingredient, then it may have been that the mold concentration was below the limit of detection at the initial counts and then the population grew during product incubation. Therefore, sourcing high-quality ingredients that meet specifications for low microbial counts is highly encouraged.

As no hummus sample processed at 87.8°C had mold growth, regardless of microbial treatment, the results suggest that a fill temperature of 87.8°C or higher is required to eliminate mold that may have been introduced from the packaging equipment or environment. Without any preservatives, the high fill temperature is required to eliminate the mold population (discussed further in Section 3.7). If natamycin is included in the formulation as a preservative, then a fill temperature of 82.2°C or higher has shown evidence to be sufficient to eliminate mold. If a combination of potassium sorbate and sodium benzoate is used, then a fill temperature as low as 76.7°C has been indicated to be sufficient to eliminate mold. Although mold is not a concern for safety with this hummus product, it is a measure of quality that is easily identified by consumers and thus should be controlled by the best methods possible.

3.4 Microbial Enumeration – Lactic Acid Bacteria

Initial lactic acid bacteria (LAB) counts were analyzed from thermally treated hummus samples within 12 hours of the jars reaching room temperature. None of the samples had any growth on MRS plates of lowest dilution (10^{-1}), regardless of fill temperature or antimicrobial

treatment. This result of no growth was expected from the lack of LAB growth in refrigerated hummus at pH 4.2 (Chapter 2, Section 3.2.2). After incubation at 35°C for 10 days, no LAB growth was observed on MRS plates of the lowest dilution (10^{-1}) for all samples. As the pH of all samples was maintained during incubation, it is further unlikely that LAB were growing in the hummus samples. It is concluded that LAB growth is inhibited in citric-acetic hummus thermally processed at 76.7°C or higher.

3.5 Microbial Enumeration – Total Plate Counts

Initial total plate counts (TPC) were analyzed from thermally treated hummus samples within 12 hours of the jars reaching room temperature. As would be expected post-thermal treatment, almost all samples had counts less than 1 log CFU/g hummus, with the only exception being samples from the control 82.2°C batch (**Figure 3.1**). Only bacteria were enumerated on the plates, being that no mold or yeast was present as previously discussed. The control 82.2°C batch had significantly higher ($p < 0.05$) initial TPC than the 76.7°C and 87.8°C control batches, but as it was the first trial completed, the difference may have been due to human error that was corrected with increased practice in the pilot plant. Even with the higher total counts, the control 82.2°C batch counts were below 2 log CFU/g, which is a suggested maximum level of microbial counts in shelf-stable foods.

After 10 days of incubation at 35°C, TPC were enumerated from the incubated samples and are presented in **Figure 3.1**. From the samples that had mold growth (**Table 3.4**), much higher TPC were observed, as would be expected. All other samples that did not exhibit mold growth had TPC of approximately 1 log CFU/g or less, which were only bacterial colonies (**Figure 3.1**). Other than the mold-spoiled samples, only the control 87.8°C and natamycin 82.2°C samples had a statistically significant increase in TPC ($p < 0.05$) after incubation

compared to initial counts from the same treatment. However, the increases are less than 1 log CFU/g for control 87.8°C and less than 0.5 log CFU/g for natamycin 82.2°C, which are not significant differences from a food safety or food quality perspective. Thus, the observed TPC increase is not of concern in the control 87.8°C and natamycin 82.2°C samples.

Although there were bacteria present in samples from all nine treatments, commercially shelf-stable foods do not have to be free of any microorganisms. According to 21 CFR Part 114.8E, commercially sterile foods should have undergone a thermal process that renders the food free of microorganisms capable of reproducing under normal storage conditions and free of viable pathogens. By this definition, viable non-pathogenic microorganisms can be present, but they should not grow (Dryer & Deibel 1992). The bacterial colonies that were present after product incubation were likely spore-formers, but they were not identified as pathogens and they were not multiplying significantly from the initial levels measured.

Therefore, applying the recommended criterion of <2 log CFU/g for microbial counts in shelf-stable foods, six of the thermal process-antimicrobial treatments could be considered shelf-stable: no antimicrobial at 87.8°C, natamycin at 82.2°C and 87.8°C, and potassium sorbate + sodium benzoate at 76.7°C, 82.2°C, and 87.8°C.

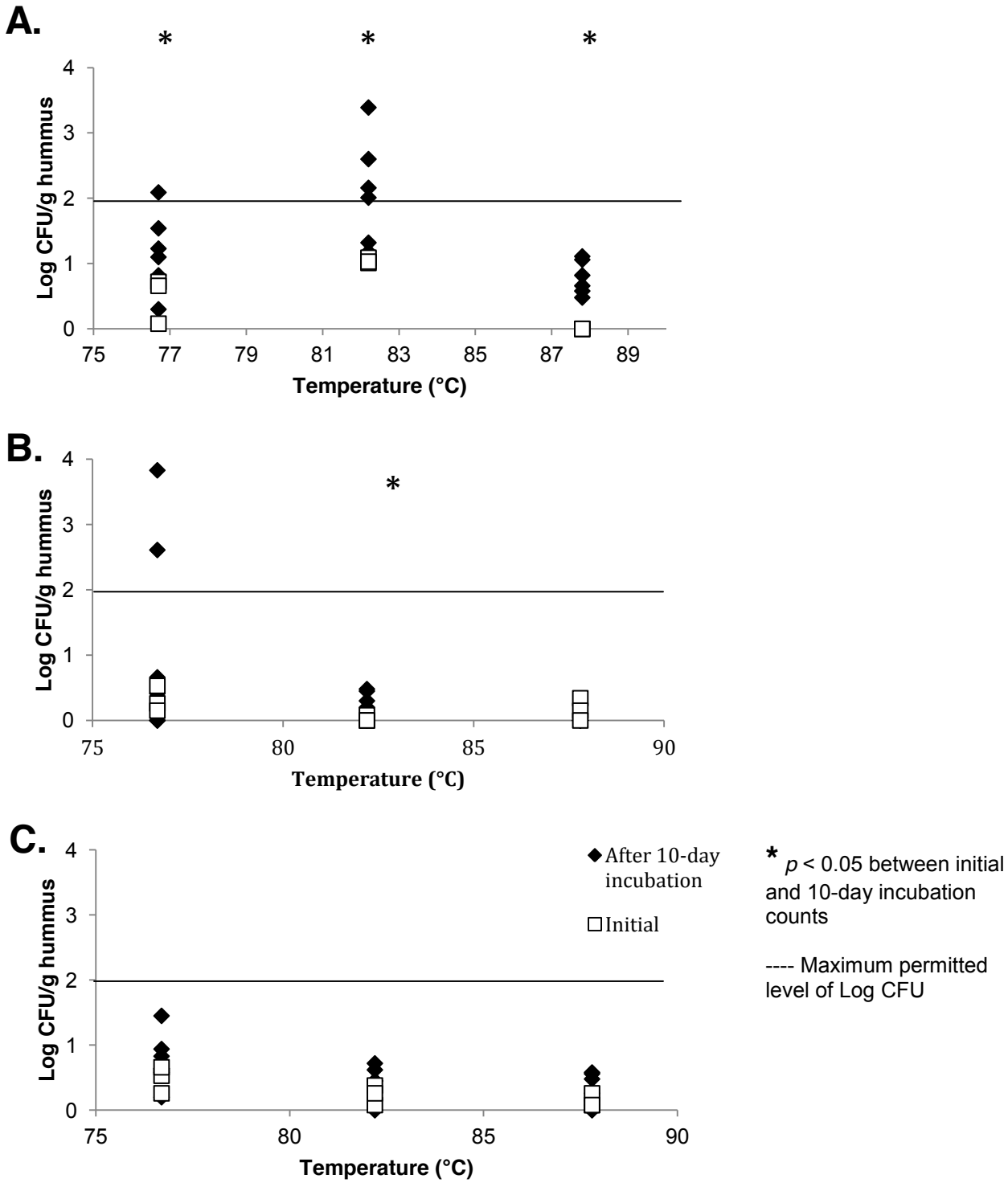

Figure 3.1. Total plate counts of thermally processed, acidified hummus samples, with A) No antimicrobial (control), B) 20 ppm Natamycin, and C) 0.1% Potassium Sorbate + 0.1% Sodium Benzoate

3.6 Lethality Values

The calculated lethality values for the three fill temperatures are presented in **Table 3.5.** According to Pflug (1998), the minimum lethality required for commercial sterilization of a product at pH 4.2 is 2.5 minutes at 93.3°C with a z-value of 8.9°C. None of the hot-fill hold processes achieved this lethality at any of the three temperatures, neither in the center nor at the side of the jar. Even if the hold times were extended to 10 minutes, the lethality of 2.5 minutes was not reached.

Table 3.5. Lethality values of acidified hummus processed at 76.7°C, 82.2°C, and 87.8°C and conditions to achieve shelf stability

	Accumulated Lethality, 5 min $F_{93.3°C}$, z = 8.9°C (Mean ± SD)		Accumulated Lethality, 10 min $F_{93.3°C}$, z = 8.9°C (Mean ± SD)		Conditions required for shelf stability
Fill Temperature	Center of Jar	Side of Jar	Center of Jar	Side of Jar	Antimicrobial treatment
76.7°C	0.10 ± 0.01^{a}	0.03 ± 0.03^{a}	0.18 ± 0.02^{a}	0.04 ± 0.04^{a}	Potassium sorbate + sodium benzoate
82.2°C	0.30 ± 0.08^{a}	0.09 ± 0.13^{a}	0.51 ± 0.16^{a}	0.11 ± 0.15^{a}	Potassium sorbate + sodium benzoate OR natamycin
87.8°C	1.24 ± 0.31^{b}	0.41 ± 0.45^{a}	2.00 ± 0.53^{b}	0.51 ± 0.55^{a}	No antimicrobial required

Values in the same column with different letters are significantly different ($p < 0.05$).

Despite not achieving the required lethality of 2.5 minutes, six of the treatments (as discussed previously) still produced a hummus product free of pathogens, as processing conditions exceeded those required to eliminate acid-adapted bacteria (Usaga et al. 2014), and free of microorganisms capable of reproducing under storage conditions. All three treatments processed at 87.8°C were successful, including the control hummus without antimicrobial. This suggests that the combination of citric and acetic acids conferred additional microbial growth control, as the process would have otherwise not eliminated the microorganisms of concern by

heat processing alone. The proposed hurdle of acetic acid addition was indeed successful at 87.8°C.

At 76.7°C and 82.2°C, the lethality values in the center of the jar were significantly lower than those achieved at 87.8°C ($p < 0.05$). The control hummus had mold growth after incubation when hot-filled at both 76.7°C and 82.2°C, indicating that the lethality conferred was not sufficient to sterilize the glass jar and lid from environmental contamination. The natamycin hummus, however, did not have mold growth at 82.2°C, demonstrating that the combined hurdles of acetic acid addition with natamycin produced a shelf-stable hummus at lower temperatures than could otherwise be achieved. The potassium sorbate + sodium benzoate hummus did not have mold growth at either 76.7°C or 82.2°C, indicating that this formulation was the most successful hurdle combination to achieve shelf stability at lower temperatures.

Therefore, although the required equivalent lethality of 2.5 minutes was not achieved, the combination of citric and acetic acids in the acidified hummus was sufficient to achieve shelf stability at a hot-fill temperature of 87.8°C or higher. The addition of natamycin to the citric-acetic hummus achieved shelf stability at an even lower temperature of 82.2°C or higher, while the addition of potassium sorbate and sodium benzoate achieved shelf stability at 76.7°C or higher. These results indicate the success of the combined hurdles of acidification with preservatives to process a shelf-stable hummus at hot-fill temperatures below the established recommendations.

3.7 Long-term Storage Study

All jars in storage at 18°C were of normal appearance at two months of storage. These observations support the results of the 35°C incubation study in which no jars had abnormalities after 10 days of incubation. Jars will be visually inspected again after four months of storage.

3.8 Recommended Processing Parameters

For a hummus sample to be considered shelf-stable, we applied the criteria of a) no mold growth observed after incubation at 35°C for 10 days, and b) total plate counts less than 2 log CFU/g initially and after incubation at 35°C for 10 days. Considering the microbial counts previously presented, there were six successful trials of formulation and processing combinations that produced a shelf-stable citric-acetic hummus at pH 4.2 with the hot-fill hold process: filling at 76.7°C with potassium sorbate plus sodium benzoate; filling at 82.2°C with natamycin OR potassium sorbate plus sodium benzoate; and filling at 87.8°C with no antimicrobial, natamycin, OR potassium sorbate plus sodium benzoate.

These six formulation and processing parameter combinations are recommended for production of a shelf-stable hummus. The use of a continuous, scraped-surface heat exchanger is also recommended to minimize equipment fouling and limit evaporation of water from the product. A steam kettle with a scraped surface agitator will also work well for batch production. These are much shorter processing times and lower processing temperatures than recommended by Amr and Yaseen (1994) for retorting hummus to produce a shelf-stable canned product.

With natamycin hummus and potassium sorbate + sodium benzoate hummus, there are options of temperatures that can be used, and it would be up to the manufacturer to choose one for processing. The decision may be based on organoleptic differences that could arise from processing the hummus at a higher temperature. As no significant difference was found in viscosity among the heat-treated hummus at any of the three temperatures, the hummus texture would be expected to be the same regardless of fill temperature. Flavor differences could occur during thermal treatment, but the degree of such differences would vary based on formulation

and additional ingredients. Consumer evaluations with the finished product may help discern if the differences are acceptable.

One benefit of choosing a lower processing temperature may be lower operational costs and less product loss during come-up time. The trials conducted at higher fill temperatures required more steam and more time to bring the hummus up to temperature. Thus, processing at 82.2°C or 76.7°C could be advantageous from a cost-savings standpoint. Additionally, smaller producers may have equipment that can process at 76.7°C but not at 82.2°C, making the use of potassium sorbate + sodium benzoate a clear choice to help achieve a shelf-stable product.

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CHAPTER 4
DRIED APPLE PRODUCT FROM AUTUMN CRISP VARIETY: PROCESS DEVELOPMENT AND CONSUMER ACCEPTABILITY

1. INTRODUCTION

Apples are one of the most important agricultural commodities worldwide, ranking 18th in the top global commodities based on production by weight in 2012 (FAOSTAT 2013). The United States is the world's second largest producer of apples after China, having produced over 4.1 million metric tons in 2012 (FAOSTAT 2013). Apples are well known for their nutritional and health benefits, ranking second among fruits in highest total phenolic content and antioxidant activity (Sun et al. 2002) and being associated with lower risks of colorectal cancer, lung cancer, and cardiovascular disease (Boyer & Liu 2004, Jedrychowski et al. 2010). In the United States, approximately 68% of the apple crop is for fresh consumption, with the remaining 32% being processed into juice, canned goods, dried, and frozen products (USDA 2012). As of 2010, only 5.7% of processed apples were used in drying applications, despite tremendous growth of the dried fruits & nuts snack market, which increased 36.1% in sales from 2007 to 2012 (Dobre-Chastain 2012). Drying fruits is also advantageous in extending shelf-life, reducing transportation weight, and minimizing storage requirements (Sagar & Kumar 2010).

Convective hot air drying of apple wedges without any additives or additional processing steps results in browning of the apple flesh due to enzymatic and nonenzymatic browning activities. With enzymatic browning, the endogenous enzyme polyphenol oxidase (PPO) reacts with phenolic compounds upon exposure to oxygen to form *o*-quinones, which leads to polymerization of brown pigments called melanins (Lozano 2006). Nonenzymatic browning occurs via Maillard browning reactions, in which free amine groups and reducing sugars react to ultimately form brown pigments called melanoidins (Lozano 2006). As color and appearance

expectations are major influences in consumer acceptability of a product (Lawless & Heymann 2010), processing regimes that reduce browning and improve appearance are of great interest. The use of sulfites effectively inhibits both forms of browning, but consumer sensitivity to sulfites has limited its use and requires labeling on products containing greater than 10 ppm sulfites (Rupp 2003). Dried fruit manufacturers desiring a "clean label" are in search of alternative options to sulfites that will still maintain the fresh color of the fruit. Multiple studies have investigated the use of pre-drying acid-dipping treatments or steam-blanching apple wedges (Beveridge & Weintraub 1995, Bolin & Steele 1987, DiPersio et al. 2009, Krokida et al. 2000, Mastrocola et al. 1996) to inhibit or inactivate PPO activity during drying. However, these methods increase processing costs and time and may negatively impact product flavor.

An alternative is to select low-browning apple cultivars for drying purposes. The varietal Autumn Crisp (formerly NY-674) developed by breeders at the New York State Agricultural Experiment Station is very popular due to its high ascorbic acid content and low PPO content (Lee & Smith 1995), resulting in low browning after cutting. The Autumn Crisp variety has been studied for applications in the fresh, minimally processed apple market, but its use and consumer preference as a dried product has not been investigated. Furthermore, research on consumer willingness-to-pay (WTP) and marketing aspects has largely focused on fresh apples, (Péneau et al. 2006, Rickard et al. 2013, Yue & Tong 2011), and as dried fruits become more popular as snacks, more information is needed for this product category. This study aims to 1) develop a processing regime of Autumn Crisp apples in convective drying applications without additives or additional processing steps to achieve a clean label, minimally processed dried product, and 2) investigate the consumer acceptability and WTP of the Autumn Crisp product compared to two commercial dried apple products, one with and one without sulfites.

2. MATERIALS AND METHODS

2.1 Sourcing of Materials

Fresh Autumn Crisp apples from the 2012 harvest were obtained from DeMarree Farms (Williamson, NY) and kept in cold storage (1.7°C) at the New York State Agricultural Experiment Station (NYSAES, Geneva, NY) until use. Commercial dried apple products were purchased from a local supermarket (Geneva, NY). Chemical reagents were purchased from EM Science (Merck KGaA, Darmstadt, Germany) or Fisher Scientific (Pittsburgh, PA). All reagents were of analytical grade.

2.2 Processing

All processing was completed in the Fruit and Vegetable Processing Pilot Plant at NYSAES (Geneva, NY). Preliminary processing studies found no significant difference in browning between Autumn Crisp apples that were pre-blanched and dipped in citric acid compared to Autumn Crisp apples that were not pretreated (data not shown). Thus, the chosen processing regime did not include blanching or dipping prior to drying. Autumn Crisp apples were removed from storage, washed in cold water, and then peeled, cored, and sliced into 16 wedges per apple using a two-stage corer-slicer machine (F.B. Pease, Rochester, NY). Wedges were dried on stainless steel trays in a food dehydrator (TSM Products, Buffalo, NY) at 65.5°C for two and a half hours, medium fan speed, and then at 57.2°C for three hours, high fan speed, to a targeted water activity of 0.6 to ensure shelf-stability. The drying temperature was decreased for the later hours as previous researchers have found that moisture diffusion is the primary mechanism for moisture movement in the final stage of drying apples, requiring lower temperatures (Doymaz 2009). Dried apple wedges were then cooled to room temperature and stored in low-density polyethylene plastic bags at 18°C until analysis and sensory evaluation.

2.3 Water Activity, pH, Titratable Acidity, Soluble Solids, Moisture Content

Both commercial dried apple products and the newly developed Autumn Crisp dried product were analyzed for physicochemical parameters. All measurements were completed in triplicate. Water activity measurements were completed using an AquaLab water activity meter Model Series T3 (Decagon Devices, Pullman, WA) at 25°C. To prepare the samples for pH, TA, and soluble solids measurement, dried apple wedges were finely chopped and blended with water (1:10 w/v). Measurements for pH were completed using a ROSS Orion Sure-flow pH electrode (Thermo Scientific, Waltham. MA). Titratable acidity (TA) was determined with an automated Mettler Toledo G20 Compact Titrator (Columbus, OH) using 0.1N NaOH to titrate to pH 8.2. Results were expressed as grams of malic acid per 100 g of dry product. Soluble solids were measured at 20°C using a Leica Auto Abbe Refractometer (Allendale, NJ) and expressed as grams sucrose per 100 g dry product. Moisture content was determined with the A&D Moisture Analyzer Model MX-50 (Tokyo, Japan) at a fixed temperature of 95°C and ended once the rate of weight loss reached 0.02 g/min. Results were expressed in percent moisture.

2.4 Instrumental Texture Analysis

Texture of the dried apple wedges was analyzed using the TA-XT2 Plus unit (Stable Microsystems, Godalming, UK) with a knife blade probe. The probe test speed and penetration depth were 2 mm/sec and 10 mm, respectively. The maximum force (N) was measured on four places per dried apple wedge to indicate hardness of the samples. Eight wedges were analyzed per product variety, and mean values were reported.

2.5 Instrumental Color Analysis

Color of the dried apple wedges was analyzed by reflectance with an UltraScan VIS Colorimeter (HunterLab, Reston, VA), which provided color coordinates on the Hunter L*, a*,

and b* color scale. L* is an index of lightness, ranging from 0 = black to 100 = white. The a* and b* axes have no numerical limit, with positive a* values indicating red, negative a* values indicating green, positive b* values indicating yellow, and negative b* values indicating blue. The colorimeter was standardized with a white standard tile and a black box reference. Triplicate measurements were made for each wedge, and six wedges were analyzed per product.

2.6 Sulfite Content Determination

To quantify the concentration of SO_2 used in the commercial sulfited product, sulfite content was determined following AOAC Method 963.20 (AOAC International 2000). The dried apple wedges were finely chopped and blended with water (1:5 w/w). The fruit slurry was centrifuged at 16500 rpm (32530 G relative force) for 30 min in a Sorvall RC-5B Refrigerated Centrifuge (Dupont Instruments). The supernatant was collected and a 5 mL aliquot was mixed with 1 mL 0.5 M NaOH in a 50 mL volumetric flask. Subsequently, 1 mL 0.25 M H_2SO_4 and 10 mL sodium tetrachloromercurate were added, and the solution was brought to volume with water. A 0.4 mL aliquot of solution was added to a test tube containing 1 mL of the color reagent, *p*-rosaniline, and followed by 2 mL addition of 0.015% (v/v) formaldehyde. The tubes were vortexed and held for 30 min at 22°C. Absorbance was read at 550 nm with a UV-Vis spectrophotometer (Genesys 10S, Thermo Scientific, Waltham, MA) against a reagent blank. The SO_2 content was reported as milligrams per kilogram (ppm). The CWOS product and the AC product were also tested using the same procedure.

2.7 Consumer Acceptability Test

A consumer acceptability test was conducted at the Cornell Sensory Testing Facility in Ithaca, NY to determine the sensory acceptability of three dried apple products: the newly developed Autumn Crisp product (AC), a commercial product with sulfites (CWS), and a

commercial organic product without sulfites (CWOS). Panelists were recruited from the Cornell University campus via flyers and email, were 18 years of age or older, and were asked not to participate if they had a sulfite sensitivity. A total of 102 panelists completed the acceptability test. The panelists were first presented with a "concept statement" describing the product they were going to sample (**Table 4.1**) and were asked to evaluate the statement using a 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). Then the panelists were asked to taste and evaluate the sample using the 9-point hedonic scale to assess the following attributes: flavor, color, shape of wedge, texture, and overall liking. After tasting evaluation, the panelists were asked to indicate how much they would be willing to pay for a six-ounce package of the sampled product, given the current market price of $3.29 and a display of the package size. The questionnaire ended with demographic questions (age, gender, education, student status, frequency of apple and dried fruit consumption) to take into account panelists' heterogeneity (see sample questionnaire in Appendix B). Panelists were also asked to rate the importance of the following attribute claims when making a purchasing decision: all-natural, no artificial preservatives, organic, and locally sourced. All samples were served at room temperature (20°C) and were presented to panelists in a random order, labeled with three-digit random codes. Panelists were presented with samples in individual testing booths next to the sample preparation area, equipped with serving windows and computers for questionnaire presentation and data collection using Compusense® 5 software.

Table 4.1 Preference of concept statements presented during consumer acceptability test

Dried Apple Product	Concept Statement	Mean Response (±SD)
Commercial with sulfites	100% apple, contains sulfites	5.45 (±1.55)[c]
Commercial without sulfites	100% organic apple, no sulfites/additives	6.44 (±1.92)[b]
Autumn Crisp	100% NY apple, no sulfites/additives	7.07 (±1.37)[a]

n = 102 for each product

[a-c]Values with different letters are significantly different from each other ($p < 0.05$)

2.8 Statistical Analysis

Compusense® 5 software was used throughout the course of the sensory acceptability study. The software was used to create the electronic questionnaire, present questionnaires to panelists according to the Williams balanced design (Carpenter & Lyon, 2000), and to collect and analyze all data. Acceptability of the sensory attributes, demographic responses, and willingness-to-pay data were analyzed using crosstabulation, percentage crosstabulation, and summary statistics (counts, medians, means and standard deviations). One-way analysis of variance (ANOVA) and Tukey's HSD were used to assess if there was a significant difference in acceptability scores and willingness-to-pay values among the three samples. JMP statistical software version 9.0 (SAS Inc, Cary, NC) was used to develop a multiple linear regression model to identify determinants of consumers' willingness-to-pay for each product. ANOVA, Kruskal-Wallis, and Tukey's HSD were used to analyze for significant differences in instrumental texture and color measurements among the three samples, and these tests were performed using JMP statistical software version 9.0. Statistical significance was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Water Activity, pH, Titratable Acidity, Soluble Solids, Moisture Content, and Sulfite Concentration

The results of physicochemical analyses and sulfite concentration for all three dried apple products are listed in **Table 4.2**. The higher A_w and moisture content observed for the CWS product was expected, as the antimicrobial effects of sulfites enable higher water content in the dried product without mold growth (Labbe & Nolan 2009). Water activity mean values differed significantly from each other among the three products ($p < 0.01$). The soluble solids contents of all three products were within the reported range of other dried apples (Lavelli & Vantaggi 2009).

The pH values of the CWOS and AC products are comparable and in the expected range for apple products, while the pH of the CWS is markedly higher. The higher pH of CWS is most likely due to the apple variety chosen for drying by the manufacturer, which may have been a low-acid apple variety.

The sulfite concentration of the CWS product is in the range of reported values for other dried apple products preserved with sulfites (ETS Laboratories 2011). Sulfite levels of the CWOS and AC products were below detectable limits (< 3 ppm), as expected, given that no sulfites were added.

Table 4.2 Instrumental physicochemical analyses of dried apple products (Mean ± SD)

Dried Apple Product	A_w	Moisture Content (%)	pH	TA (g malic/ 100g dried product)	Soluble solids (g/100g dried product)	Sulfite concentration (ppm)
Commercial without sulfites	0.552 ± 0.001	14.48 ± 0.58	3.49 ± 0.03	2.98 ± 0.49	71.80 ± 0.53	< 3†
Commercial with sulfites	0.689 ± 0.003	21.63 ± 0.70	4.26 ± 0.04	1.27 ± 0.05	71.72 ± 4.48	651 ± 82
Autumn Crisp	0.603 ± 0.004	16.59 ± 0.66	3.62 ± 0.07	4.17 ± 0.25	75.13 ± 2.95	< 3†

†Concentration was lower than detection limit of 3 ppm by standard curve

3.2 Instrumental Texture Analysis

Dried apple wedge texture differed significantly ($p < 0.01$) among the three product varieties tested (**Figure 4.1**). The CWOS product resulted in the highest maximum force, indicating a harder texture, which was significantly greater ($p < 0.05$) than the maximum force measurements for either the CWS or AC products. Lower water activity has been associated with increased textural hardness of dried figs, dried apples, and prunes in other studies (Beveridge & Weintraub 1995, Farahnaky et al. 2010, Gabas et al. 2002), and our results confirm these findings. Maximum force measurements between the CWS and AC products were not significantly different ($p > 0.05$), implicating that the two products are similar in texture by

instrumental analysis despite differing significantly in A_w values. Differences in sensory-perceived texture are discussed in Section 3.4.

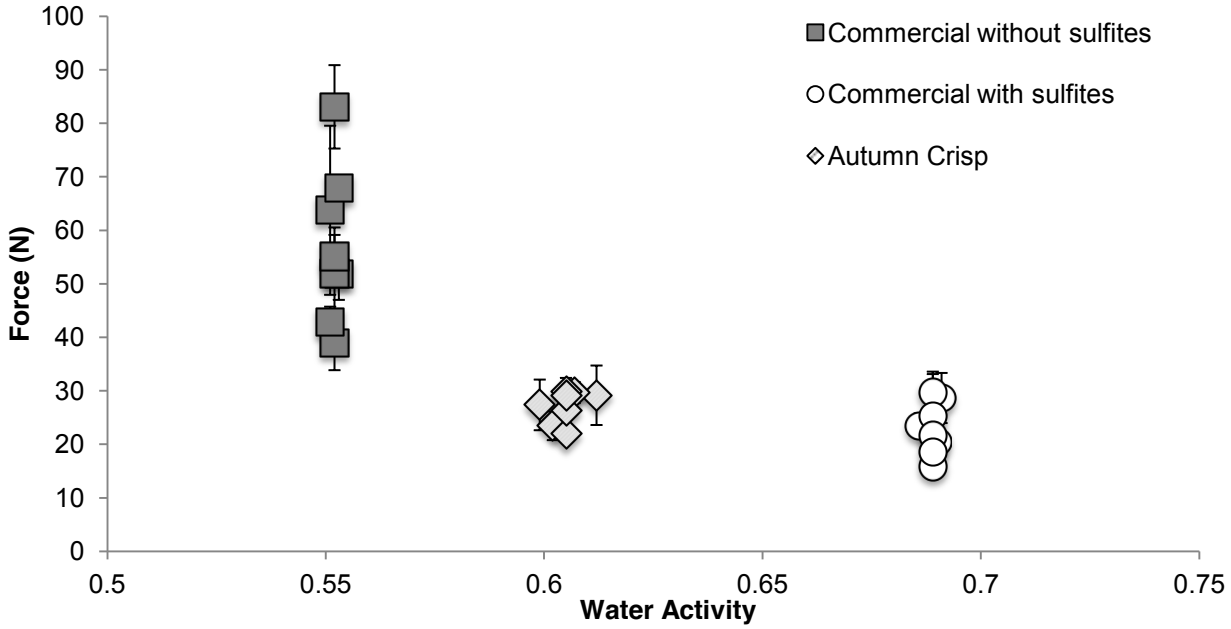

Figure 4.1 Instrumental texture hardness of sample dried apple wedges, measured in force. *Error bars represent standard deviation of mean (n = 8).*

3.3 Color Analysis

The colorimetric analyses of the three products are illustrated on three axes of L*, a*, and b* in **Figure 4.2**. The colorimetric parameter for whiteness, L*, differed significantly in value among the three products (ANOVA, $p < 0.01$). Of all three products, CWOS was the darkest from browning (mean L* value of 70.01), while AC was the lightest (mean L* value of 87.49). For the colorimetric parameter of red-green, the CWOS product had the highest a* value (mean of 11.91), which was significantly greater than values of the CWS and AC products ($p < 0.01$). An increase of redness is interpreted as signifying occurrence of browning reactions (Krokida et al. 2000), further indicating that the CWOS product had a higher degree of browning than the other two products. In terms of the yellowness index (b*), only the values between the CWS and

CWOS differed significantly ($p < 0.05$), with the CWS product being the highest in yellow color (mean b* value of 31.94) and the CWOS product being the least (mean b* value of 28.03). The b* value of the AC product did not differ significantly from either of the two other products. Yellowness of the dried wedges is most likely due to the variety of apple, and as the apple variety of the commercial products are unknown, these differences could not be controlled. Consumer-perceived color differences determined during the sensory test are discussed in Section 3.4.

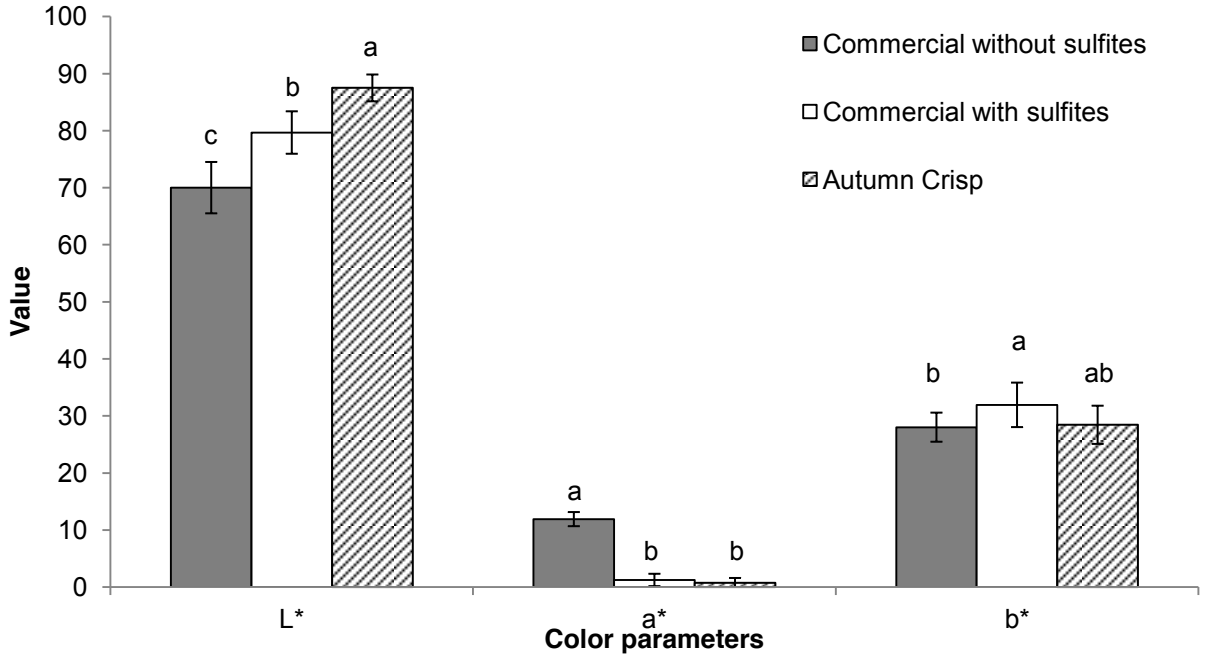

Figure 4.2 Colorimetric values of sample dried apple wedges, measured by instrumental analysis. *Error bars represent standard deviation of mean (n = 9).*
[a-c] Bars in same parameter with different letters are significantly different ($p < 0.05$)

3.4 Consumer Acceptability of Dried Apples

The results of the acceptability test for sensory attributes of the three products are presented in **Figure 4.3**. The AC sample received the highest overall liking score of 6.56, although there was no statistically significant difference ($p > 0.05$) in liking between this product and the CWS product (score of 6.37). The overall liking score of the CWOS sample was

significantly lower ($p < 0.05$) than the other two products. Across all sensory attributes (color, flavor, texture and shape of wedge), the AC and CWS samples received significantly higher ($p < 0.05$) acceptability scores than the CWOS sample. These results suggest that the AC and CWS products were equally acceptable to consumers, as there was no statistically significant difference of scores in any sensory category between the two products.

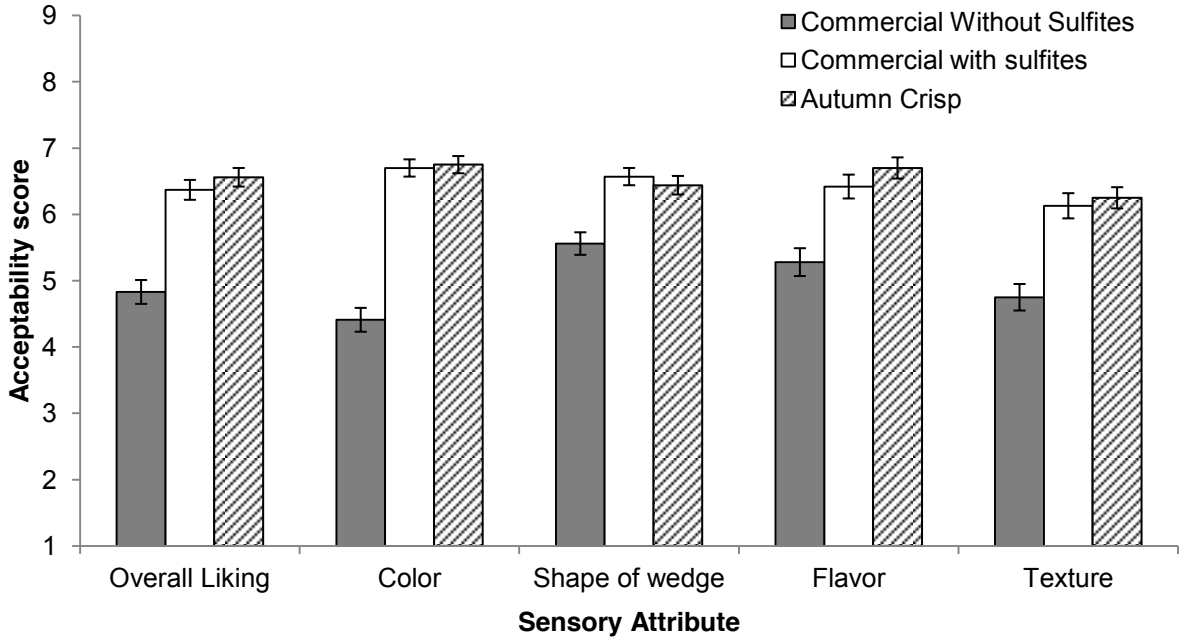

Figure 4.3 Mean acceptability scores of sensory attributes for sample dried apple wedges, assessed by *n*=102 panelists. *Error bars represent standard error*.

Interestingly, it is important to note that the AC sample received an acceptability score on color (6.75) that was not significantly different from the CWS sample score the same attribute (6.70). This indicates that the low-browning properties of the Autumn Crisp variety resulted in a dried apple product appearance that was as acceptable as the appearance of a sulfited product. These consumer sensory scores concur with the instrumental color analysis, in which the CWS and AC samples did not differ significantly in their a* values. In contrast, the CWOS sample scored the lowest across all attributes, particularly in the color attribute (score of 4.41). The low

color score is also related to the colorimetric analysis of the CWOS sample, which had a* value being much higher and indicating greater browning. The combination of consumer acceptability scores and colorimetric results suggest that the Autumn Crisp variety is statistically significantly more acceptable as a dried apple product, without the use of sulfites, compared to the apple varieties currently being used in commercial product applications without sulfites.

Consistent with the instrumental texture analysis, texture acceptability scores did not differ between the CWS and AC samples. In contrast, the texture acceptability score was significantly lower ($p < 0.05$) for the CWOS sample. Although some panelists commented that the CWS sample felt very "moist," its texture score suggested that it is as acceptable as the AC sample. Together, the instrumental and panelist sensory evaluation results indicate that dried apple products that are softer in texture, in conjunction with having a higher A_w, are more acceptable to consumers than harder, drier products.

A correlation matrix (**Table 4.3**) was computed to determine the effects of other sensory attributes on the overall liking scores. All attributes were highly positively correlated with overall acceptability, with flavor and texture having the strongest correlation ($R^2 = 0.79$ and $R^2 = 0.76$, respectively). Consumers have cited flavor as an important attribute for fresh apple quality (Péneau et. al 2006, Rickard et. al 2013), and it is not surprising that its high importance applies to dried apple products as well. Other researchers have found also texture to play a significant role in consumer acceptability, particularly in perceived quality, and it is often more important than flavor (Lawless & Heymann 2010). This further suggests that optimizing the moisture content and water activity of a dried fruit product is necessary to maximize consumer acceptability. From panelists' free responses for improvements to the products, there was a definitive split between those who believed that all the products should have been crispier and

those that thought the products were too dry and chewy (comment data not shown). Thus, it is important for the dried fruit industry to note that consumers have differing expectations for the texture quality of the products.

Table 4.3 Correlation matrix of sensory attributes of dried apple products determined in consumer acceptability test

	Overall Liking	Color	Shape of wedge	Flavor	Texture
Overall Liking	1.0000	0.6945	0.5904	0.7924	0.7635
Color		1.0000	0.5822	0.5675	0.5955
Shape of wedge			1.0000	0.5406	0.5378
Flavor				1.0000	0.7041
Texture					1.0000

3.5 Consumer Willingness-to-Pay for Dried Apples

The summary statistics for demographic data and willingness to pay (WTP) from the panelists' responses are presented in **Tables 4.4** and **4.5**, respectively. Approximately 16.7% of the panelists were between 18-25 years old, 39.2% between 26-35 years old, 19.6% between 36-45 years old, 14.7% between 46-55 years old, and 9.8% over 55 years old. Two-thirds of the panelists were female. Of the panelists, 35% were students, with the remainder being university staff, faculty, or community members. Approximately 64% of panelists consumed apples/apple products "several times a week." On average, the panelists consumed dried fruits once a week or a few times a month, and over 80% had exposure to dried apples prior to the sensory evaluation. The product attribute claims of "all-natural" and "no artificial preservatives" were rated to be of greater importance to the majority of panelists compared to "organic" or "locally-sourced" product claims.

Table 4.4 Characteristics of panelists in consumer acceptability test

Demographic Variable	Description	N	% of panelists
Age	Panelist's age in years	102	
	[1] = 18-25		16.7
	[2] = 26-35		39.2
	[3] = 36-45		19.6
	[4] = 46-55		14.7
	[5] = 55+		9.8
Gender	[0] = Male	102	33.3
	[1] = Female		66.7
Student Status	[0] = Nonstudent	102	64
	[1] = Student		36
Education	Level of education completed	102	
	[1] = Some high school		1.0
	[2] = High school diploma		7.8
	[3] = Some college		5.9
	[4] = 2/ 4-year college degree		18.6
	[5] = Some graduate school		20.6
	[6] = Graduate degree		46.1
Frequency of apple consumption	[1] = Several times/week	102	63.7
	[2] = Once/week		21.6
	[3] = Once/month		9.8
	[4] = Less than once/month		4.9
Consumer of dried fruits	[0] = No	102	14.7
	[1] = Yes		85.3
Frequency of dried fruit consumption	[1] = Several times/week	87	18.4
	[2] = Few times/week		25.3
	[3] = Once/week		9.2
	[4] = Few times/month		33.3
	[5] = Once/month		6.9
	[6] = Less than once/month		6.9
Prior exposure to dried apples	[0] = No	102	18.6
	[1] = Yes		81.4
Importance of "all-natural" claim	[1] = Not important	102	4.9
	[2] = Somewhat important		23.5
	[3] = Important		30.4
	[4] = Very Important		27.5
	[5] = Extremely important		13.7
Importance of "no artificial preservatives" claim	[1] = Not important	102	5.9
	[2] = Somewhat important		22.6
	[3] = Important		27.5
	[4] = Very Important		28.4
	[5] = Extremely important		15.7
Importance of "organic" claim	[1] = Not important	102	26.5
	[2] = Somewhat important		32.4
	[3] = Important		26.5
	[4] = Very Important		8.8
	[5] = Extremely important		5.9
Importance of "locally sourced" clam	[1] = Not important	102	19.6
	[2] = Somewhat important		24.5
	[3] = Important		31.4
	[4] = Very Important		16.7
	[5] = Extremely important		7.8

Of the three samples, panelists responded that they would be willing to pay the most for the AC samples, with a mean willingness-to-pay (WTP) of $3.13. This value is not significantly different ($p > 0.05$) from the panelists' mean WTP for the CWS sample ($3.09). However, panelists valued both of these samples statistically significantly higher ($p < 0.01$) than the CWOS samples (mean WTP value of $2.92).

Table 4.5 Willingness-to-pay (WTP) for 6-oz. package of dried apple products after sensory evaluation

Dried apple product	N	Mean WTP ($)	Standard Deviation
Commercial without sulfites (CWOS)	102	2.92[a]	0.38
Commercial with sulfites (CWS)	102	3.09[b]	0.40
Autumn Crisp (AC)	102	3.13[b]	0.39

[a,b] Values with different letter are significantly different ($p < 0.01$)

A multiple linear regression model was developed using ordinary least squares (OLS) estimates to explain the variation of WTP among panelists while incorporating random effects to account for the panel nature of the data. The final model is presented in **Table 4.6**. Type of product, flavor of product, panelist status (student or not a student), and importance of all-natural claims were statistically significantly correlated with WTP ($p < 0.05$). Gender, age, level of education, frequency of apple consumption, frequency of dried fruit consumption, and prior exposure to dried apples were not statistically significant variables ($p > 0.05$) and therefore were not included in the final model. As other sensory attributes (color, shape of wedge, texture, overall liking) were highly correlated with flavor, only flavor was incorporated into the model to minimize effects accruing to multicollinearity. Of the attributes, flavor had the strongest correlation with WTP and the lowest p-value ($p < 0.01$).

Table 4.6 Willingness-to-pay estimates for dried apple products using ordinary least squares random effects model

Parameter	Coefficient estimate	Standard Deviation
Intercept	2.360**	0.105
$Product_0$ (CWOS)	-0.059**	0.020
$Product_2$ (AC)	0.038*	0.019
Student $Status_1$ (1 = student)	0.100**	0.031
Flavor liking score	0.084**	0.008
Importance score of all-natural product claim	0.065*	0.027

*Indicates significance at 5% level ($p < 0.05$)
**Indicates significance at 1% level ($p < 0.01$)

From construction of the WTP regression model, it was found that 56.19% of the variance in WTP is due to differences among panelists. The remaining 43.81% of the variance is due to the factors discussed above. Overall, the R-squared value (0.76) indicates that the model has strong explanatory power of the WTP for these dried apple products. Results in **Table 4.6** show that the intercept estimate is $2.36, and the coefficients for the AC and CWOS products tested ranged from -$0.06 to $0.04, which are both statistically significant (CWS products is the excluded dichotomous variable and thus its price is included in the intercept). These results show that the average WTP for AC products is $0.04 higher relative to CWS products, while the WTP for CWOS products is lower by $0.06 in comparison to CWS products. The results also suggest, as expected from the high correlation of flavor with acceptability, that with each unit increase in flavor liking, the WTP increases by $0.08. Moreover, the WTP increases if the panelist was a student and if the panelist placed greater importance on all-natural product attributes. It is somewhat surprising that students were found to have higher WTP than nonstudents (approximately $0.10), and that no significant interactions were found between student status and any other variable. However, student panelists also responded with higher liking scores to the product concepts presented at the beginning of the test (**Table 4.1**) compared to the non-student panelists ($p < 0.05$). This suggests that the product concepts tested in this

study were overall more appealing to students, and thus they may have been willing to pay more in general. Another possible explanation could be that confounding variables not considered may impact consumer WTP (e.g., valuation of healthy eating, percentage of income spent on food and money typically spent on snacks).

Our results indicate that consumers find Autumn Crisp dried apple products to be as acceptable as sulfited dried products and are also willing to pay more for this product, as it would include all-natural claims. The marketing significance of our results is illustrated in **Table 4.7**, which presents a comparison of WTP for the three products using the parameter estimates from the regression model in **Table 4.6**. For example, a 6 oz. package of dried apple product that does not use sulfites, is made from Autumn Crisp apples, and has an average flavor score would be valued at $3.12 by non-student consumers who have an average valuation of all-natural claims. This is 10 cents more than the same exact product that is made instead from an apple variety other than Autumn Crisp. Consumer WTP is approximately the same for sulfited products as for Autumn Crisp (difference of $0.02) if the consumer places no importance on natural claims. However, if the consumer does value all-natural claims, then they will pay a full 16 cents more for the Autumn Crisp product compared to the sulfited product.

Table 4.7 Comparison of prices based on willingness-to-pay regression model

Product Description	Consumer category	WTP ($)
Product without sulfites, not Autumn Crisp variety, average flavor score	Non-student, minimal importance of all-natural	2.88
	Non-student, average importance of all-natural	3.02
Product without sulfites, Autumn Crisp variety, average flavor score	Non-student, minimal importance of all-natural	2.98
	Non-student, average importance of all-natural	3.12
Product with sulfites, not Autumn Crisp variety, average flavor score	Non-student, minimal importance of all-natural	2.96
	Non-student, average importance of all-natural	2.96

The panelists' WTP responses also emphasize the importance of sensory attributes in consumers' purchasing decisions. Although panelists responded positively to the CWOS product concept (**Table 4.1**), upon tasting the product, their opinions and preferences changed to favor the alternative choices and they gave the lowest WTP value for CWOS. Responses to the CWS concept statement were also opposite to the panelists' final overall liking and WTP for the product. Previous researchers have found that consumers will alter preferences and WTP after tasting fresh apples that did not meet expectations of freshness or overall quality (Lund et al. 2006). When rating apples only by sensory attributes, consumers were also found to prefer the apple variety that they rated the highest in sensory attributes and were willing to pay more for this variety (Yue & Tong 2011). Our results indicate that consumer preferences of dried apple products are led by flavor, appearance, and other sensory attributes, similar to fresh fruit preferences. Moreover, sensory attributes impact the consumers' WTP more strongly than quality claims, as the flavor coefficient in the model had a stronger statistical significance than the all-natural importance coefficient.

4. PROCESSING RECOMMENDATIONS AND RESEARCH LIMITATIONS

It is possible to produce dried apples of the Autumn Crisp variety without resultant browning by simple convective drying, and no pretreatments or sulfite additions are needed to maintain the fresh color and appearance. Dried apple products made from the Autumn Crisp variety are found to be as acceptable to consumers as commercial sulfited products in terms of flavor, texture, color, and overall liking. Dried apple products that are not sulfited and have the darker brown color are significantly less acceptable. Consumer sensory evaluations are supported by instrumental color and texture analysis, indicating that higher A_w, softer texture, and lighter color are more acceptable. Willingness-to-pay for dried apple products depends on

the product type and product flavor, with additional variation due to a consumer's status as a student or non-student and how much they value all-natural claims. Consumers are willing to pay the same amount for Autumn Crisp dried apple products as commercial products with sulfites, indicating a strong market potential for this apple variety in the dried product category.

There are limitations to the model developed from this study. All panelists currently live in the central upstate New York area, which may not reflect the consumer attitudes of the national population. Panelists were also recruited from a university town and therefore may have different educational exposure than the general population. The products tested were chosen to be representative of samples in the dried apple market, but some differences do exist, as these are agricultural products that differ from season to season. Future research of consumers from other market areas may help further define the sensorial aspects and marketing claims of dried apple products that impact willingness-to-pay.

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CHAPTER 5
CONCLUSIONS & RECOMMENDATIONS FOR FUTURE WORK

The findings of the hummus projects and dried apple wedges project demonstrate that small-scale producers can successfully manufacture products with natural ingredients and minimal processing to satisfy consumer expectations. From the results of the hummus shelf life studies and the thermal processing trials, formulation and processing guidelines can be written for the production of refrigerated and shelf-stable hummus that meets the scientific basis for safe and high-quality foods. Specifically, the application of multiple hurdles (acidification, addition of preservatives, processing temperature) improved the microbial stability of hummus and was shown to extend the shelf life for a longer period of time than either hurdle alone. From instrumental analysis and consumer evaluations, it was determined that dried apple wedges from Autumn Crisp apples could be made without chemical preservatives and still be as acceptable as sulfited commercial products.

Acidification of hummus with citric and acetic acids to pH below 4.6 ensured the inhibition of *Clostridium botulinum* while also conferring antimicrobial effects from the acetic acid. The combination of these two acids was the most successful of the acid systems tested to inhibit microbial growth, both bacterial and fungal. The addition of 20 ppm natamycin further improved the microbial stability of citric-acetic hummus with regards to mold growth, demonstrating fungicidal activity and resulting in a shelf life of up to 22 weeks at 5°C. This shelf life was significantly longer than the 6-10 weeks achieved with potassium sorbate or sodium benzoate, confirming that natamycin, a natural preservative, performs better than these chemical preservatives. Sensory discrimination tests with consumers confirmed that there is no perceivable difference between hummus with citric acid and hummus with citric-acetic acids. Therefore, the combination of citric and acetic acids with natamycin could be successfully

incorporated into an existing hummus formulation without significant changes to flavor, making it possible for hummus manufacturers to extend shelf life while using natural ingredients.

Although the hummus products were determined to be microbially stable for 22 weeks at 5°C, it is possible that other quality changes could occur during that time period, thereby limiting the shelf-life. Given the large percentage of olive oil inclusion, lipid oxidation is a potential concern that would result in rancid flavors and aromas (Bendini et al. 2009), which would be unacceptable from a quality standpoint. Refrigeration will decrease the oxidative reactions but will not stop them completely. Future research on the rate of lipid oxidation and rancidity in refrigerated citric-acetic hummus with natamycin would be helpful in assessing the organoleptic quality of the product throughout its shelf life. More extensive sensory tests over shelf life would also be beneficial to determine how flavor and aroma may change during 22 weeks of refrigeration and at what point these changes become unacceptable to consumers.

Beyond refrigeration, applying both hurdles of acidification and antimicrobial addition also improved stability of shelf-stable hummus products and permitted lower processing temperatures. Utilizing the hot-fill hold method with 6 oz. glass jars and holding for 5 minutes, the following formulation combinations and fill temperatures were successful in producing a shelf-stable product:

1. Citric-acetic hummus acidified to pH 4.2, hot-filled at 87.8°C
2. Citric-acetic hummus acidified to pH 4.2 + 20 ppm natamycin, hot-filled at 87.8°C
3. Citric-acetic hummus acidified to pH 4.2 + 1000 ppm potassium sorbate + 1000 ppm sodium benzoate, hot-filled at 87.8°C
4. Citric-acetic hummus acidified to pH 4.2 + 20 ppm natamycin, hot-filled at 82.2°C

5. Citric-acetic hummus acidified to pH 4.2 + 1000 ppm potassium sorbate + 1000 ppm sodium benzoate, hot-filled at 82.2°C
6. Citric-acetic hummus acidified to pH 4.2 + 1000 ppm potassium sorbate + 1000 ppm sodium benzoate, hot-filled at 76.7°C

The hot-fill hold method is feasible for small-scale producers to execute, and processing at lower temperatures of 76.7°C or 82.2°C will reduce the time and energy required to produce a shelf-stable hummus product.

Thus, the concept of mild preservation hurdle technology by Leistner (2000) was achieved for this formulation of hummus with the chosen packaging. The parameters discussed above can be used as guidelines for writing scheduled processes; however, each product and package is slightly different. If a hummus manufacturer wishes to incorporate an ingredient that could alter the pH or use a different size/material of container, then the suggested temperatures and treatment would have to be reconsidered. Future thermal processing trials with different containers or hummus pH values may be beneficial to broaden the knowledge of the available processing options.

As previously discussed, the change in viscosity or flavor of thermally-processed hummus could impact consumer acceptability. No sensory evaluations were completed with this part of the study because the hummus formulations were designed for scientific replication and standardization, not for sensorial likeability. Evaluations of hummus that has added ingredients for optimal flavor at pH 4.2 would be more beneficial to understand consumer acceptability of a thermally-processed shelf-stable product. Additionally, questions that target consumers' opinions of the texture and flavor may help determine which processing temperature is preferred for maintaining organoleptic qualities of that specific product.

The future of hurdle technology in hummus production is still an area of discovery. As previously mentioned, high pressure processing (HPP) with hummus has been applied for extension of refrigerated shelf life. While HPP is already being used with some hummus manufacturers in the industry, future research into its effects on specific quality attributes like viscosity and flavor may be of interest. It may also be useful to combine HPP with natural preservatives like natamycin to further extend the shelf life. However, HPP is a very costly operation to install, and most companies would have to sub-contract the hummus production to a co-packer that has a HPP unit. Such investments may not be possible for small-scale entrepreneurs, making HPP a non-viable option. Furthermore, bacterial spores are highly pressure-resistant, thereby requiring a combination of heat and high pressures to eliminate pathogens like *Clostridium botulinum* (Rastogi et al. 2007). Thus, production of shelf-stable hummus products with HPP requires further research to determine its feasibility under different conditions such as type and amount of acid addition, and final pH values.

Natamycin was clearly identified in this study as a successful preservative in hummus against mold growth. Although granted GRAS status in the U.S. and approved for use in cheese, it is not explicitly approved for use in hummus. In our trials, natamycin was not used above the current legal limit (20 ppm) and had no adverse taste effects. As the safety and stability of natamycin is already proven, we hope that the evidence of natamycin's effectiveness against mold growth in hummus will support petitions for natamycin to have its FDA approval extended to hummus applications as well. It may also be of interest to further quantify the bacterial inhibition observed from natamycin in this study to identify its potential as an antibacterial agent.

By simple convective drying and no pretreatments or sulfite additions, dried apples of the Autumn Crisp variety had no resultant browning and maintained their fresh color and appearance

over storage. From the results of instrumental analysis and consumer sensory evaluations, it was determined that dried apple products made from the Autumn Crisp variety are as acceptable to consumers as commercial sulfited products in terms of flavor, texture, color, and overall liking. Non-Autumn Crisp dried apple products that are not sulfited and have a darker brown color are significantly less acceptable. Willingness-to-pay for dried apple products was found to be dependent on the product type and product flavor, with additional variation due to a consumer's status as a student or non-student and how much they value all-natural claims. Consumers are willing to pay the same amount for Autumn Crisp dried apple products as commercial products with sulfites, indicating a strong market potential for this apple variety in the dried product category. Additional research on dried apple products in larger consumer markets may help further define the impact of other demographic variables on consumers' willingness-to-pay and product acceptability. Future work on drying applications with other low-browning apple varieties would also be useful to determine the marketability of those products as well.

The formulation and processing recommendations concluded from this project can be of assistance for determining the scheduled processes of hummus products and dried apple products for both small and large producers looking to use natural ingredients. This will be particularly helpful for the work of the Food Venture Center, where extension work is focused on the needs of small-scale food entrepreneurs. By applying the acidification, preservative, thermal processing, and sensory evaluation knowledge from these studies, we will help the manufacturers achieve their desired shelf life while still providing a high-quality product.

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APPENDIX

A. Sample triangle test ballot for hummus sensory discrimination test

Panelist 1 **Date: 26 February 2014**

Hummus Triangle Test

Instructions: You will receive two different sets of 3 hummus samples. For each set of samples, two of the samples are the same, and one is different. Taste each of the three samples **in the order presented** from left to right. Indicate which sample is different by circling the code of the different sample on the score sheet below. Be sure to rinse your mouth with water prior to beginning and between each sample.

Set #1

156 273 684

Notes:

_____

Set #2

873 286 439

Notes:

_____

B. Sample ballot for consumer acceptability test of dried apple wedges

Acceptability Test

In this test you will evaluate the characteristics of Premium Dried Apple Wedges.
You will taste 3 samples. Please check the box that correspond to your answer based on your opinion of the product. For each sample you will evaluate 5 characteristics and indicate your opinion for each.
Sample Number: _____

Overall Liking:

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Color:

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Shape of Wedge:

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Flavor:

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Texture:

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Any additional comments:

In retail outlets, a 6 oz. package of dried apple wedges sells for $3.29 (see sample bag of 6 oz. on table). How much would you be willing to pay for a 6 oz. package of this sample #? (approximately 4 servings per package)

- Less than $2.60
- $2.60 - $3.28
- $3.29
- $3.30 - $4.00
- More than $4.00

B. Sample questionnaire for dried apple wedges consumer acceptability test, continued

Please indicate your age in years: 18-25 _____ 26-35 ____ 36-45 ____ 46-55 ____ Over 55 ____

Please indicate your gender: Male ____ Female _____ Prefer not to answer ____

Please indicate the level of education have you completed: Some high school ___ High school diploma ___ Some college ___ 4-year college degree ___ Some graduate school ___ Graduate degree ___

Please indicate your position at Cornell University: Student __ Staff ___ Faculty ___ Academic appointment ___ Other ___

How often do you consume apple products (e.g. fresh apples, apple juice, applesauce, etc)? Less than once a month ___ 1-2 times per month ____ 1-2 times per week ___ 3-4 times per week ___ 5-6 times per week ___

Do you consume dried fruits? Yes ___ No ___

If you answered "yes" to the previous question, how often do you consume dried fruit products? Less than once a month ___ 1-2 times per month ____ 1-2 times per week ___ 3-4 times per week ___ 5-6 times per week ___

Have you eaten dried apples before? Yes ___ No ___

Please rate the importance of the following attributes when you are making food purchase decisions:

(1 = Not important, 5 = Extremely important)

All-natural	1	2	3	4	5
No artificial preservatives	1	2	3	4	5
Organic	1	2	3	4	5
Locally-sourced ingredients	1	2	3	4	5